ORIGINAL ARTICLE

Airflow Limitation and Endothelial Dysfunction

Unrelated and Independent Predictors of Atherosclerosis

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Abstract

Rationale: Lower FEV_1 is associated with increased prevalence of atherosclerosis; however, causal mechanisms remain elusive.

Objectives: To determine if systemic endothelial dysfunction mediates the association between reduced FEV_1 and increased atherosclerosis.

Methods: Brachial artery endothelial function, pulmonary function, coronary artery calcium, and carotid plaque were assessed in 231 Pittsburgh SCCOR (Specialized Centers for Clinically Oriented Research) study participants; peripheral arterial endothelial function, pulmonary function, and coronary artery calcium were assessed in 328 HeartSCORE (Heart Strategies Concentrating on Risk Evaluation) study participants.

Measurements and Main Results: Lower FEV_1 was independently associated with increased atherosclerosis in both cohorts (per 25% lower % predicted FEV_1: odds ratio [OR], 1.76; 95% confidence interval [CI], 1.30–2.40; $P < 0.001$ for carotid plaque in SCCOR participants) (per 25% lower % predicted FEV_1: OR, 1.35; 95% CI, 1.02–1.77; $P = 0.03$ for coronary artery calcium in HeartSCORE participants). Similarly, reduced endothelial function was independently associated with increased atherosclerosis in both cohorts (per SD lower endothelial function: OR, 1.30; 95% CI, 1.01–1.67; $P = 0.04$ for carotid plaque in SCCOR participants) (per SD lower endothelial function: OR, 1.38; 95% CI, 1.09–1.76; $P = 0.008$ and OR, 1.41; 95% CI, 1.07–1.86; $P = 0.01$ for coronary artery calcium in SCCOR and HeartSCORE participants, respectively). However, there was no association between endothelial dysfunction and FEV_1, FEV_1/FVC, low-attenuation area/visual emphysema, and diffusing capacity in SCCOR participants, and between endothelial dysfunction and FEV_1 or FEV_1/FVC in HeartSCORE participants (all $P > 0.05$). Adjusting the association between FEV_1 and atherosclerosis for endothelial dysfunction had no impact.

Conclusions: Endothelial dysfunction does not mediate the association between airflow limitation and atherosclerosis. Instead, airflow limitation and endothelial dysfunction seem to be unrelated and mutually independent predictors of atherosclerosis.

Keywords: chronic obstructive pulmonary disease; cardiovascular disease; endothelium

Lower FEV_1 is independently associated with greatly increased risk of coronary and carotid atherosclerosis (1–3). Furthermore, each 10% lower percent predicted FEV_1 has been associated with 28% increase in cardiovascular mortality after accounting for tobacco exposure, age, and other vascular disease risk factors (4). Nonetheless, causal mechanisms linking airflow limitation with atherosclerosis remain elusive.

It has been hypothesized that systemic endothelial dysfunction mediates the association between FEV_1 and atherosclerosis (i.e., lower FEV_1 is associated with lower systemic endothelial

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At a Glance Commentary

Scientific Knowledge on the Subject: Reduced FEV_1 is associated with greatly increased risk of atherosclerosis. It has been hypothesized that systemic endothelial dysfunction mediates this association (i.e., reduced FEV_1 results in more systemic endothelial dysfunction that then contributes to atherosclerosis).

What This Study Adds to the Field: Contrary to this hypothesis, we report that systemic endothelial dysfunction does not mediate the association between reduced FEV_1 and increased atherosclerosis. Instead, reduced FEV_1 and endothelial dysfunction are unrelated and mutually independent predictors of atherosclerosis. These findings were robust to different assessments of endothelial function (brachial artery flow–mediated dilation vs. digital reactive hyperemia), atherosclerosis (carotid vs. coronary), and were present in two unrelated cohorts that included more than 500 participants in aggregate. Further examination of the interrelationship among airflow limitation, endothelial dysfunction, and atherosclerosis is required.

function that then leads to atherosclerosis) (Figure 1A) (5, 6). Prior studies have examined the relationship between expiratory airflow and endothelial dysfunction but have produced inconsistent results (6–11). Because these studies did not assess atherosclerosis *per se*, it remains unknown if endothelial dysfunction mediates the association between FEV_1 and atherosclerosis.

We assessed expiratory airflow, systemic endothelial dysfunction, and carotid/coronary atherosclerosis in 231 participants recruited from the Pittsburgh chronic obstructive pulmonary disease (COPD) SCCOR (Specialized Centers for Clinically Oriented Research) study and 328 participants in the HeartSCORE (Heart Strategies Concentrating on Risk Evaluation) cohort. We hypothesized that reduced systemic endothelial function would mediate the association between FEV_1 and atherosclerosis (Figure 1A).

Methods

Study Cohorts

The Pittsburgh SCCOR study enrolled 758 current or prior smokers (>10 pack-years) between the age of 40 and 79 years that resided in and around Allegheny County (12). Those with a predominant restrictive pattern on spirometry, prior thoracic surgery, and vascular or cardiac events in the preceding year were excluded (12). During two SCCOR follow-up visits, we consecutively enrolled 231 participants into a substudy without applying additional selection criteria besides absence of surgical history or vascular abnormality that would preclude assessment of carotid atherosclerosis or affect brachial artery blood flow. These 231 participants were similar to the remaining cohort besides fewer pack-years of smoking (46.4 ± 21.7 vs. 61.5 ± 35.7; $P < 0.001$) and higher diffusing capacity of carbon monoxide ($D_{L_{CO}}$) (77.1 ± 16.2 vs. 71.1 ± 18.9% predicted; $P = 0.001$).

HeartSCORE is an ongoing community-based prospective cohort study that initially enrolled 2,000 participants. Eligibility criteria included age 45–75 years, residence in the greater Pittsburgh metropolitan area, ability to undergo baseline and annual follow-up visits, and absence of known comorbidities expected to limit life expectancy to less than 5 years (13). Tobacco exposure was not required for inclusion. In the current study, we included 328 participants who had pulmonary function, endothelial function, and coronary artery calcium scores assessed as part of various substudies. These participants were similar to the remaining cohort besides being older (61.4 ± 6.8 vs. 58.6 ± 7.6 yr; $P < 0.001$), more frequently men (62.8% vs. 39.1%; $P < 0.001$), and having better endothelial function (reactive hyperemia index 0.76 ± 0.47 vs. 0.66 ± 0.41; $P < 0.001$).

Presence of airflow obstruction ($FEV_1/FVC < 0.70$) was not required for inclusion in either cohort. Both cohorts were approved by the University of Pittsburgh Institutional Review Board and written informed consent was obtained from each participant.

Endothelial Function

In the SCCOR cohort, endothelial function was assessed by sonographic measurement of brachial artery flow-mediated dilation per standardized guidelines (14). In the HeartSCORE cohort, peripheral arterial endothelial function was assessed by measuring digital reactive hyperemia using the EndoPAT system (Itamar Medical Ltd., Caesarea, Israel) per published recommendations (15, 16). Digital reactive hyperemia reflects the endothelial function of both medium and small arteries in the upper extremity.

Greater flow-mediated dilation and reactive hyperemia indicate better (healthier) endothelial function. Additional methodologic details are provided in the online supplement.

Carotid Plaque

Transverse and longitudinal B-mode images of the common carotid, carotid bulb, internal carotid, and external carotid arteries were obtained (GE 9L probe, 3–10 MHz, GE Healthcare, Tokyo, Japan; and GE Vivid 7 Dimension ultrasound, GE Vingmed Ultrasound, Horten, Norway). Plaque was defined per standardized guidelines (i.e., as a focal structure protruding ⩾0.5 mm into the arterial lumen or ⩾1.5 times the thickness of the surrounding intima media) (17). The total number of plaque in both arteries was classified into three categories (none, 1–3, and >3).

Coronary Artery Calcium

In the SCCOR cohort, coronary artery calcium was assessed using non-ECG-gated chest computed tomography (CT) scans. Calcium scores from non-ECG-gated CT have excellent correlation with scores from gated cardiac CT, and independently predict coronary vascular events and mortality (18–22). Coronary artery calcium was scored using the Weston method (20). Weston scores increase from 0 to 12 with increasing amounts of coronary calcium. Scores were divided into four categories because of their skewed distribution (0 = none, 1–3 = mild, 4–7 = moderate, 8–12 = severe). These categories correspond to the four categories of Agatston coronary artery calcium scores (0, 1–100, 101–400, >400) with sensitivity of 100% and specificity of greater than 85% (20). Interobserver and intraobserver agreement on calcium scores was excellent (both $\kappa > 0.90$; *see* online supplement). Coronary artery calcium scoring could not be performed in five participants because of intracoronary stents

and in six participants because of device artifacts.

In the HeartSCORE cohort, ECG-gated electron beam tomography was performed as described previously (23). Coronary artery calcium scores were calculated by the method of Agatston and divided into four categories (0, 1–100, 101–400, >400) (24).

Pulmonary Function, Diffusing Capacity, and Emphysema

In the SCCOR cohort, prebronchodilator and post-bronchodilator spirometry was performed per American Thoracic Society recommendations and adjusted to standard population-derived predicted values (post-bronchodilator values were used for all analyses) (25). Residual volume/total lung capacity ratio was assessed by full-body plethysmography. Diffusing capacity was assessed as the single-breath $D_{L_{CO}}$ (26). Emphysema severity was assessed by density mask analysis of chest CT scans using a −950 (or −910) Hounsfield unit cutoff (i.e., low-attenuation area [LAA] % −950 and −910) and by semiquantitative visual score that increased from 0 to 5 with increasing severity of emphysema (27, 28). In the HeartSCORE cohort, prebronchodilator spirometry was performed in compliance with 2005 American Thoracic Society/European Respiratory Society Spirometry Standards and adjusted to standard population-derived predicted values (25).

Cardiovascular Risk Factors

Hypertension was defined as a measured systolic blood pressure greater than 140 mm Hg or diastolic blood pressure greater than 90 mm Hg, or patient report of a prior diagnosis of hypertension, or use of antihypertensive medication. Hyperlipidemia and diabetes were defined based on patient report, or use of medication for hyperlipidemia or diabetes. In the HeartSCORE cohort, fasting glucose levels were performed and a level greater than 126 mg/dl was an additional criteria to define diabetes. Fasting lipid levels were also performed in HeartSCORE (VAP; Atherotech, Birmingham, AL) and were included in data analyses. Smoking history was self-reported.

Statistical Analyses

FEV_1 was defined at the independent variable, carotid/coronary atherosclerosis as the dependent variable, and endothelial function as the mediator. A mediator was defined as per Baron and Kenney: a variable may be called a mediator to the extent that it accounts for the relation between the independent (FEV_1) and dependent variable (carotid/coronary atherosclerosis) (29). To establish mediation, the following conditions must hold: (*1*) FEV_1 must be associated with endothelial function (Figure 1A, *a*); (*2*) endothelial function must be associated with atherosclerosis (Figure 1A, *b*); and (*3*) when endothelial function is accounted for the previously significant relationship between FEV_1 and atherosclerosis should no longer be significant, with the strongest demonstration of mediation occurring when the relationship becomes null (29). These relationships were tested using regression models (ordinal logistic regression for category of carotid/coronary atherosclerosis and linear regression for FEV_1 and endothelial function). When ordinal models were used, the proportional odds assumption was tested using likelihood ratio tests that returned nonsignificant *P* values.

Established cardiovascular risk factors and variables associated with coronary or carotid atherosclerosis with *P* less than 0.15 in univariate analyses were adjusted as covariates in all models. These included age, sex, smoking status, pack-years of smoking, hypertension, hyperlipidemia, and diabetes in the SCCOR cohort; and age, sex, race, waist/hip ratio, hypertension, diabetes, hyperlipidemia, smoking status, total cholesterol level, low-density lipoprotein cholesterol level, and serum triglycerides in the HeartSCORE cohort.

In the HeartSCORE cohort, assessments of pulmonary function, endothelial function, and atherosclerosis were performed during different study visits. Accordingly, additional analyses were performed to ensure that time differences between assessments did not confound results. Analyses were performed using STATA MP Version 13.1 (StataCorp, College Station, TX). A two-tailed *P* less than 0.05 was defined as statistically significant.

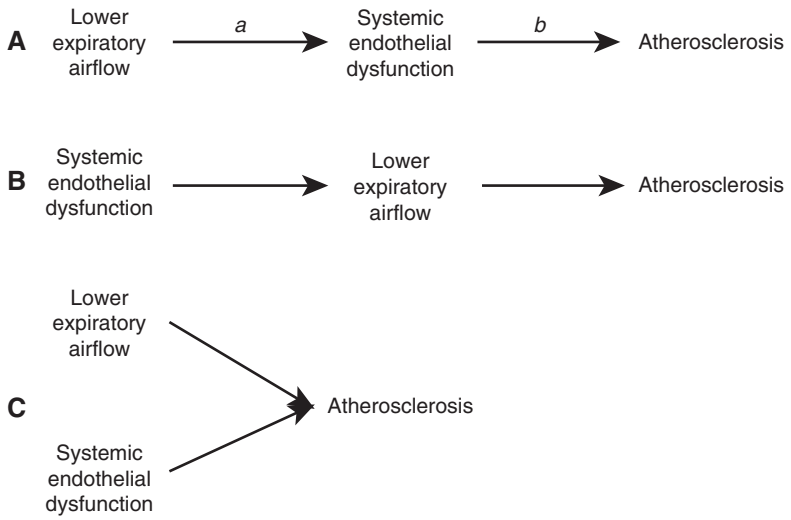

Figure 1. (*A–C*) Some of the possible relationships among airflow limitation, endothelial function, and atherosclerosis. In scenario *A*, lower expiratory airflow is associated with systemic endothelial dysfunction (*arrow a*), and systemic endothelial dysfunction is associated with atherosclerosis (*arrow b*).

Results

The study included 231 Pittsburgh SCCOR study participants and 328 HeartSCORE study participants. SCCOR participants were 68.1 ± 6.2 years in age, 53.7% male, and predominantly non-Hispanic white (Table 1). All were current or prior smokers (>10 pack-years) and 42.6% had an FEV_1/FVC less than 0.70 (Table 1). Carotid plaque and coronary artery calcium was present in most (Table 1). Multiple established cardiovascular risk factors were associated with increased plaque and coronary artery calcium including age, sex, tobacco exposure, hypertension, diabetes, and hyperlipidemia (Table 1).

Table 1. Characteristics of Pittsburgh SCCOR Participants with Varying Severity of Carotid and Coronary Atherosclerosis Summarized as Mean (SD) for Continuous Variables and Proportion for Categorical Variables

Variable	All	Number of Carotid Plaque			*P* Value*	Extent of Coronary Artery Calcification				*P* Value*
		None	1–3	>3		None	Mild	Moderate	Severe	
n (%)	231	87 (37.6)	92 (39.9)	52 (22.5)		30 (13.6)	36 (16.4)	60 (27.3)	94 (42.7)	
Demographics										
Age, yr	68.1 (6.2)	66.8 (6.0)	68.7 (6.8)	69.1 (5.1)	**0.03**	63.4 (3.7)	66.6 (4.9)	68.6 (6.6)	69.8 (6.1)	**<0.001**
Male, %	53.7	46.0	53.3	67.3	**0.02**	20.0	35.3	57.6	68.5	**<0.001**
Non-Hispanic white, %	98.3	97.7	98.9	98.1	0.36	96.7	97.1	100.0	97.8	0.43
Risk factors										
Hypertension, %†	62.8	54.0	60.9	80.8	**0.003**	26.7	55.9	71.2	68.5	**0.001**
Diabetes mellitus, %‡	12.1	4.6	15.2	19.2	**0.007**	6.7	8.8	10.2	16.3	0.09
Hyperlipidemia, %§	58.9	49.4	56.5	78.8	**0.002**	26.7	55.9	59.3	70.6	**<0.001**
BMI, kg/m^2	28.3 (4.2)	28.4 (4.1)	28.1 (4.4)	28.3 (4.1)	0.88	27.6 (4.3)	27.7 (4.3)	28.8 (4.5)	28.2 (4.0)	0.58
Current smoker, %	35.5	33.3	34.8	40.4	0.44	33.3	44.1	33.9	38.0	0.92
Pack-years of smoking	53.6 (28.7)	45.5 (19.7)	54.5 (27.1)	65.5 (38.7)	**<0.001**	38.6 (19.0)	48.3 (21.0)	52.6 (24.2)	61.7 (35.1)	**<0.001**
Lung disease										
FEV_1, % predicted	84.6 (21.4)	89.8 (18.0)	85.6 (21.4)	74.2 (23.2)	**<0.001**	86.3 (16.0)	91.5 (18.2)	84.0 (20.8)	81.1 (23.7)	**0.04**
FEV_1/FVC	0.68 (0.12)	0.71 (0.09)	0.68 (0.13)	0.63 (0.15)	**<0.001**	0.72 (0.12)	0.71 (0.10)	0.67 (0.12)	0.66 (0.14)	**0.01**
FEV_1/FVC <0.70 and					**0.001**					**0.02**
FEV_1 ⩾80% predicted	12.1	10.1	11.4	16.7		3.3	5.9	15.2	15.2	
FEV_1 50–79% predicted	23.7	19.0	21.6	35.4		20.0	26.5	23.7	23.9	
FEV_1 30–49% predicted	6.1	2.5	6.8	10.4		3.3	2.9	5.1	8.7	
FEV_1 <30% predicted	1.4	0.0	1.1	4.2		0.0	0.0	1.7	2.2	
Post-BD change, %	7.9 (7.9)	7.8 (7.6)	6.8 (7.2)	10.1 (9.2)	0.23	9.5 (8.8)	7.7 (6.7)	7.9 (7.9)	7.4 (8.0)	0.28
D_{LCO}, % predicted	75.7 (14.6)	75.8 (16.5)	71.5 (18.7)	69.2 (17.5)	**0.03**	72.4 (17.4)	75.8 (16.5)	71.5 (18.7)	69.2 (17.6)	**0.03**
Emphysema score	1.0 (1.3)	0.7 (1.1)	1.1 (1.3)	1.5 (1.4)	**0.001**	1.0 (1.3)	0.7 (1.0)	0.8 (1.0)	1.2 (1.4)	**0.05**
LAA%, HU −950	3.3 (6.9)	1.9 (4.0)	3.9 (7.7)	4.7 (8.6)	**0.02**	1.9 (4.4)	1.5 (2.4)	3.9 (7.2)	4.3 (8.4)	**0.04**
LAA%, HU −910	16.8 (15.0)	14.1 (12.0)	18.1 (16.7)	19.0 (16.1)	**0.05**	12.3 (11.9)	13.5 (9.7)	19.4 (14.4)	17.9 (17.8)	0.07

Definition of abbreviations: BMI = body mass index; D_{LCO} = diffusing capacity of the lung for carbon monoxide; HU = Hounsfield units; LAA% = low-attenuation area in the lung measured by density mask analysis; Post-BD change = percent change in FEV_1 after bronchodilator administration; SCCOR = Specialized Centers for Clinically Oriented Research.
Bold indicates $P < 0.05$.
*P values are from ordinal logistic regression models with category of carotid plaque/coronary calcium score as the dependent variable.
†Measured blood pressure >140 mm Hg systolic or >90 mm Hg diastolic, use of antihypertensive medication, or patient report of the diagnosis.
‡Patient report of the diagnosis or use of medication for diabetes.
§Patient report of the diagnosis or use of medication to treat hyperlipidemia.

HeartSCORE participants were on average younger (61.4 ± 6.5 vs. 68.1 ± 6.2 yr), more frequently men (62.8 vs. 53.7%), less frequently non-Hispanic white (53.7 vs. 98.3%), and less likely to have severe coronary artery calcification (11.3 vs. 42.7%) compared with those from the SCCOR cohort (Table 2). A total of 55.2% were ever smokers and 11.2% had an FEV_1/FVC less than 0.70.

Expiratory Airflow and Endothelial Function

Expiratory airflow was not associated with endothelial function in either cohort. In the SCCOR cohort, there was no association between FEV_1 and brachial artery flow-mediated dilation ($P = 0.61$). Neither adjustment for covariates nor repeating analyses in those with airflow obstruction (FEV_1/FVC <0.70), emphysema (%LAA −950 >1%), or in prior smokers alter the results. In the HeartSCORE cohort, a very weak correlation between FEV_1 and reactive hyperemia index was present (Spearman $r = 0.09$; $P = 0.09$); however, adjustment for covariates eliminated this trend ($P = 0.57$).

Endothelial Function and Atherosclerosis

Endothelial function was independently associated with increased atherosclerosis in both cohorts. In the SCCOR cohort, reduced flow-mediated dilation was associated with increased carotid plaque and coronary artery calcium in unadjusted (Figure 2A) and adjusted analysis (per SD lower flow-mediated dilation, odds ratio [OR], 1.30, 95% confidence interval [CI], 1.01–1.67, $P = 0.04$ for carotid plaque; and OR, 1.35, 95% CI, 1.02–1.78, $P = 0.03$ for coronary calcium). Similarly, in the HeartSCORE cohort, reduced reactive hyperemia index was associated with increased coronary artery calcium in unadjusted (Figure 2B), and adjusted analysis (per SD lower reactive hyperemia index, OR, 1.36; 95% CI, 1.07–1.73; $P = 0.01$).

Expiratory Airflow and Atherosclerosis

Lower FEV_1 was independently associated with increased atherosclerosis in both cohorts. In the SCCOR cohort, lower FEV_1 was associated with more carotid plaque in univariate (Figure 2A) and multivariate analyses (OR, 1.76 per 25% lower % predicted FEV_1; 95% CI, 1.30–2.40; $P < 0.001$); and with more coronary artery calcium in univariate (Figure 3A) but not in multivariate analysis (OR, 1.28 per 25% lower % predicted FEV_1; 95% CI, 0.93–1.78;

Table 2. Characteristics of HeartSCORE Participants with Varying Severity of Coronary Atherosclerosis Summarized as Mean (SD) for Continuous Variables and Proportion for Categorical Variables

Variable	All	Extent of Coronary Artery Calcification				*P* Value*
		None	Mild	Moderate	Severe	
n (%)	328	66 (20.1)	151 (46.0)	74 (22.6)	37 (11.3)	
Demographics						
Age, yr	61.4 (6.5)	59.5 (6.5)	60.2 (6.8)	63.9 (6.2)	64.2 (6.4)	**<0.001**
Male, %	62.8	51.5	56.3	78.4	78.4	**<0.001**
Race, %						**0.05**
Non-Hispanic white	53.7	54.5	45.7	68.9	54.1	
Non-Hispanic black	42.1	42.4	49.7	28.4	37.8	
Other	4.3	3.1	4.6	2.7	8.1	
Risk factors						
Hypertension, %†	70.9	66.7	68.0	73.0	86.5	**0.05**
Systolic blood pressure, mm Hg	142.8 (18.6)	140.3 (20.8)	141.2 (17.6)	145.1 (18.1)	149.0 (18.1)	**0.009**
Diastolic blood pressure, mm Hg	83.0 (9.8)	81.3 (10.2)	83.5 (10.3)	83.3 (9.3)	84.2 (7.7)	0.20
Diabetes, %‡	20.5	18.1	23.3	20.5	13.5	0.56
Fasting serum glucose, mg/dl	103.1 (27.9)	102.2 (27.1)	105.6 (32.0)	101.9 (23.6)	96.8 (16.4)	0.37
Hyperlipidemia, %§	48.2	44.0	46.4	54.1	51.3	0.22
Medication for dyslipidemia, %	26.9	22.7	22.0	37.8	32.4	**0.03**
Waist/hip ratio	0.92 (0.66)	0.92 (0.07)	0.94 (0.07)	0.94 (0.06)	0.93 (0.05)	**<0.001**
Total cholesterol level, mg/dl	207.8 (44.9)	207.3 (43.8)	213.9 (47.3)	192.7 (32.7)	213.8 (52.1)	0.24
LDL cholesterol level, mg/dl	144.6 (39.9)	142.4 (39.2)	148.2 (40.8)	135.2 (32.3)	152.7 (47.9)	0.85
HDL cholesterol level, mg/dl	46.6 (12.6)	47.0 (14.9)	46.7 (11.9)	46.6 (11.7)	45.3 (13.0)	0.58
Triglyceride level, mg/dl	141.9 (83.2)	147.8 (115.0)	149.4 (82.9)	125.8 (51.7)	128.9 (62.9)	**0.05**
Smoking status, %						0.09
Never	44.8	59.1	45.0	37.8	32.4	
Prior	45.1	34.8	43.1	51.4	59.5	
Current	10.1	6.1	11.9	10.8	8.1	
Airflow limitation						
FEV_1, % predicted	96.0 (19.9)	100.1 (17.4)	95.7 (20.7)	94.8 (19.4)	91.9 (21.6)	**0.04**
FEV_1/FVC	0.79 (0.09)	0.80 (0.09)	0.81 (0.09)	0.76 (0.10)	0.77 (0.11)	**0.009**
FEV_1/FVC <0.70, %	11.4	15.1	5.6	18.9	13.9	0.30

Definition of abbreviations: HDL = high-density lipoprotein; HeartSCORE = Heart Strategies Concentrating on Risk Evaluation; LDL = low-density lipoprotein. Bold indicates $P < 0.05$.

*P values are from ordinal logistic regression models with category of calcium score as the dependent variable.

†Measured blood pressure >140 mm Hg systolic or >90 mm Hg diastolic, use of antihypertensive medication, or patient report of the diagnosis.

‡Patient report of the diagnosis, fasting blood glucose >126 mg/dl, or use of medication for diabetes.

§Patient report of the diagnosis or use of medication to treat hyperlipidemia.

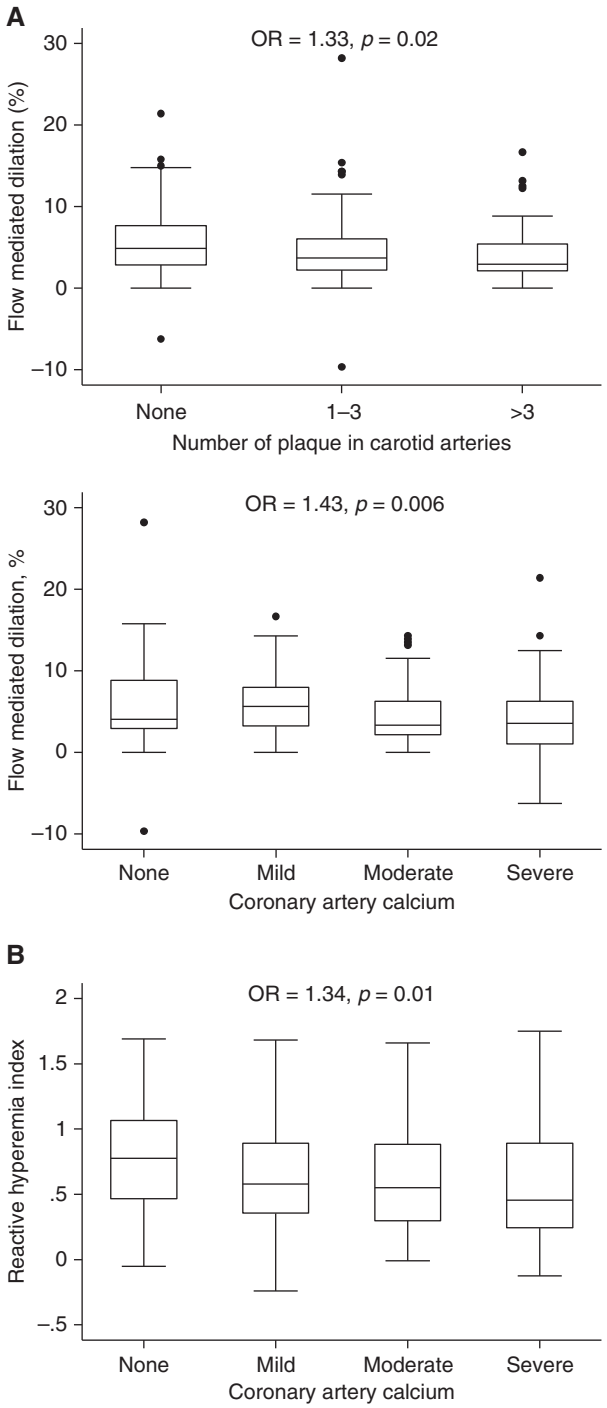

Figure 2. Lower endothelial function was associated with increased atherosclerosis in the SCCOR (Specialized Centers for Clinically Oriented Research) (n = 231) (*A*) and HeartSCORE (Heart Strategies Concentrating on Risk Evaluation) (n = 328) (*B*) cohorts. The odds ratios and *P* values are for 1 SD lower endothelial function as the independent variable and ordinal categories of carotid plaque or coronary artery calcium as the dependent variable. OR = odds ratio.

$P = 0.13$). In HeartSCORE, reduced FEV_1 was associated with more coronary artery calcium in both univariate (Figure 3B) and multivariate analysis (OR, 1.41 per 25% lower % predicted FEV_1; 95% CI, 1.07–1.86; $P = 0.02$).

Adjusting the independent associations between FEV_1 and atherosclerosis for endothelial function had no impact in either cohort (Figure 4). Therefore, endothelial function did not mediate the association between FEV_1 and atherosclerosis.

Additional Analyses

We performed several additional analyses to test the robustness of our results. First, instead of low FEV_1 leading to reduced endothelial function the opposite may be true (i.e., reduced endothelial function may lead to low FEV_1 that then leads to atherosclerosis) (Figure 1B). However, the association between reduced endothelial function and atherosclerosis was unchanged after adjusting for FEV_1 (*see* Figure E1 in the online supplement), arguing against this hypothesis.

Second, rather than FEV_1, other smoking-related pulmonary phenotypes may be associated with reduced endothelial function and atherosclerosis including airflow obstruction, hyperinflation, emphysema, or diffusing capacity. However, there was no association between brachial artery flow-mediated dilation and FEV_1/FVC ($P = 0.25$), %LAA −950 ($P = 0.95$), %LAA −910 ($P = 0.52$), visual emphysema score ($P = 0.33$), or D_{LCO} ($P = 0.50$) in the SCCOR cohort. There was a trend with residual volume/total lung capacity ratio ($P = 0.11$); however, this was weakened after adjusting for age and sex ($P = 0.57$) and other covariates ($P = 0.90$). Similarly, there was no association between reactive hyperemia index and FEV_1/FVC in the HeartSCORE cohort ($P = 0.20$). Also, adjusting for endothelial function did not impact the associations between these phenotypes and atherosclerosis, and vice versa (*see* online supplement).

Third, it is possible that endothelial dysfunction mediates the association between FEV_1 and atherosclerosis only in those with more significant lung disease. Therefore, analyses were repeated in SCCOR participants with FEV_1/FVC less than 0.70 and FEV_1 less than 80% predicted (n = 76), visual emphysema score greater than or equal to 2 (n = 61), and D_{LCO} less than 60% predicted (n = 51). In these three subgroups, the independent association between FEV_1 and atherosclerosis seemed to be stronger than in the overall cohort (per 25% lower % predicted FEV_1: in those with FEV_1/FVC <0.70 and FEV_1 <80% predicted, OR, 4.47, 95% CI, 1.77–11.27, $P = 0.002$ for carotid plaque and OR, 4.98,

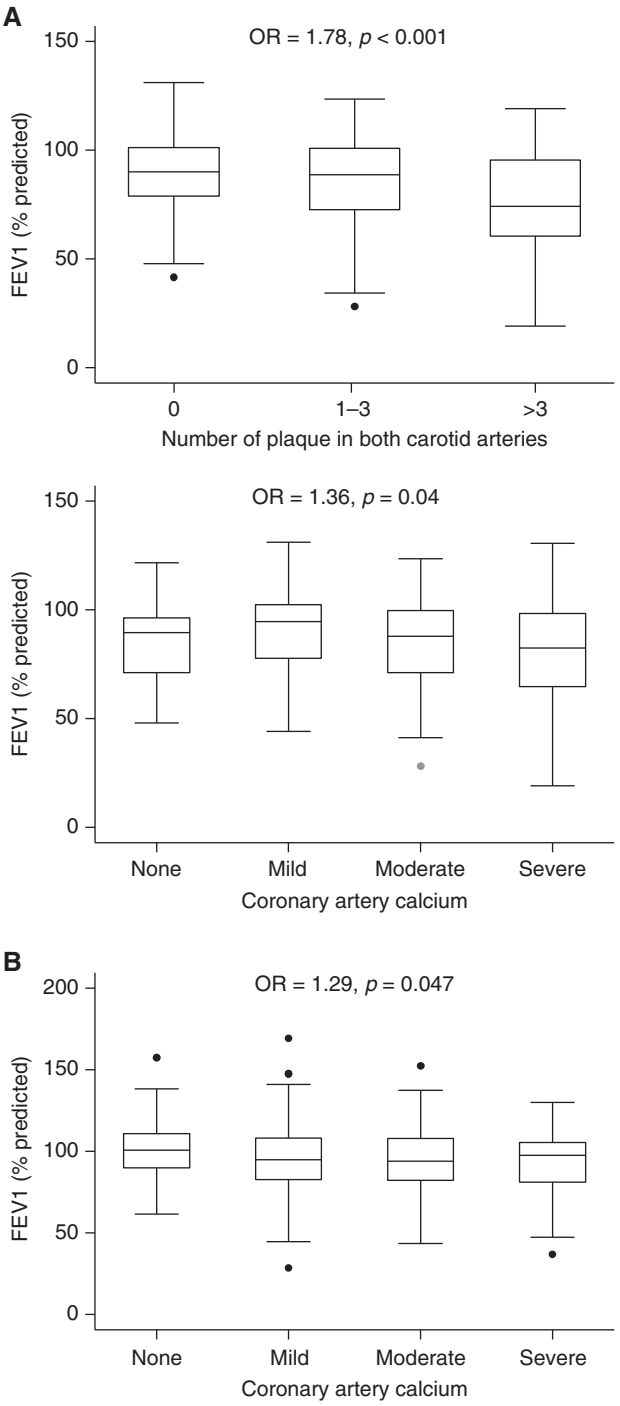

Figure 3. Lower FEV_1 was associated with increased atherosclerosis in the SCCOR (Specialized Centers for Clinically Oriented Research) (n = 231) (*A*) and HeartSCORE (Heart Strategies Concentrating on Risk Evaluation) (n = 328) (*B*) cohorts. Odds ratios and *P* values are for 25% lower % predicted FEV_1 as the independent variable and ordinal categories of carotid plaque or coronary artery calcium score as the dependent variable. OR = odds ratio.

95% CI, 1.87–13.27, $P = 0.001$ for coronary calcium) (in those with visual emphysema score $\geq$2 OR, 2.23, 95% CI, 1.25–4.32, $P = 0.008$ for carotid plaque and OR, 1.51, 95% CI, 0.87–2.61, $P = 0.14$ for coronary calcium) (in those with D_{LCO} <60% predicted OR, 2.45, 95% CI, 1.23–4.73, $P = 0.008$ for carotid plaque, and OR, 1.63, 95% CI, 0.88–3.04, $P = 0.12$ for coronary calcium). In contrast, brachial artery flow-mediated dilation was no longer associated with atherosclerosis (adjusted $P = 0.59$, 0.29, and 0.25 for carotid plaque and $P = 0.32$, 0.41, and 0.09 for coronary calcium in the three subgroups, respectively). Again, flow-mediated dilation was not associated with FEV_1 ($P = 0.85$, 0.97, and 0.82 in the three subgroups, respectively). Therefore, endothelial dysfunction was not associated with either atherosclerosis or FEV_1 in those with more significant lung disease, although sample sizes in these subgroups were notably smaller than in the overall cohort.

Furthermore, excluding participants with prior vascular events had no impact (five SCCOR participants reported prior diagnosis of myocardial infarction, four reported a diagnosis of angina, and one reported a prior stroke; two HeartSCORE participants reported angiographically diagnosed coronary artery disease, data not shown). Finally, using prebronchodilator rather than post-bronchodilator spirometry values for SCCOR participants, adjusting for time difference between assessments in the HeartSCORE cohort, adjusting for medications that may impact endothelial function, or using nonordinal rather than ordinal logistic regression did not alter the results (*see* online supplement).

Discussion

This is the first study to examine the relationship between airflow limitation, endothelial dysfunction, and atherosclerosis. We hypothesized that endothelial dysfunction mediates the association between airflow limitation and atherosclerosis (Figure 1A). However, we found that endothelial dysfunction and airflow limitation were unrelated and mutually independent predictors of atherosclerosis (Figure 1C). This finding was robust to different assessments of endothelial function (brachial artery flow-mediated dilation vs. digital reactive hyperemia), atherosclerosis (carotid sonography vs. coronary artery calcium score), and pulmonary phenotype (airflow limitation, airflow obstruction, hyperinflation, emphysema, and diffusing capacity), and was present in two unrelated cohorts.

Prior studies that have investigated the relationship between airflow limitation and

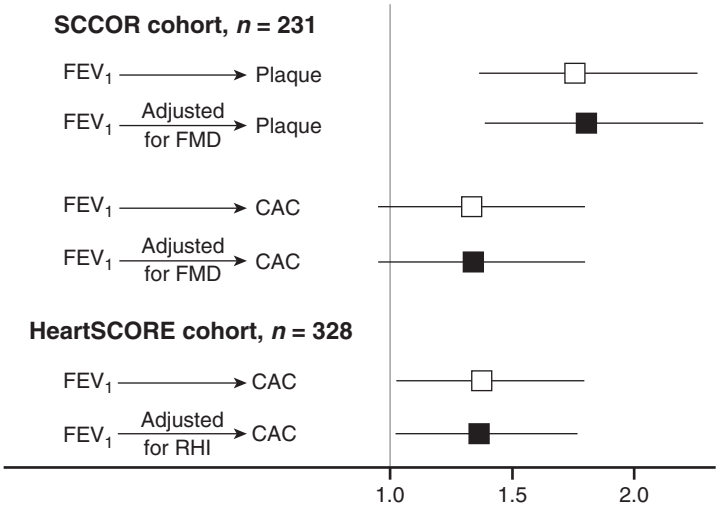

Figure 4. Adjusting the association between reduced FEV_1 and increased carotid plaque or coronary artery calcium for endothelial function had no impact (i.e., endothelial function did not explain the association between expiratory airflow and atherosclerosis). All models are fully adjusted for covariates as described in the text. CAC = coronary artery calcium; FMD = flow-mediated dilation; HeartSCORE = Heart Strategies Concentrating on Risk Evaluation; RHI = reactive hyperemia index; SCCOR = Specialized Centers for Clinically Oriented Research.

endothelial function, however, have produced inconsistent results (6–11, 30). Specifically, a study that used the gold standard method to assess endothelial function (invasive measurement of forearm vasoreactivity to intraarterial infusion of vasodilators) did not identify an association between airflow limitation and endothelial function (30). In contrast, others have reported statistically significant associations between airflow limitation and endothelial function, although with large differences in effect size (6–11). For example, there was a greater than 10-fold difference in the magnitude of change in endothelial function per unit change in FEV_1 between two studies ($\beta = 0.22$ vs. 0.019; both $P < 0.05$) (6, 10).

Differences in participant selection may have contributed to the variable results of prior studies. Some studies excluded women (8); current smokers (9, 11); those treated with statins or antihypertensive medication (6, 10); and those with a history of coronary, cerebrovascular, or peripheral artery disease (30) or heart failure (8). In one study only 13% of eligible participants met inclusion criteria (6). Although these criteria were appropriate to exclude patients with conditions known to affect endothelial function, an unintended consequence may be the limited generalizability and inconsistency of the results obtained.

In the current study, we used community-based cohorts and did not apply the selection criteria used in prior studies. Also, we confirmed the biologic validity of the endothelial function identified in our cohorts by demonstrating independent associations with atherosclerosis, unlike in prior reports. This is important because endothelial function is assessed in these studies to identify a form of vascular injury associated with atherosclerosis. Also, measurement of brachial artery flow-mediated dilation is operator and interpreter dependent, and requires a high level of skill to produce valid results.

We found that airflow limitation and endothelial function were unrelated and mutually independent predictors of atherosclerosis; however, endothelial dysfunction may still contribute to systemic vascular injury in patients with lung disease. First, endothelial dysfunction may explain the high prevalence of peripheral artery disease in patients with airflow limitation (31). Second, acute derangements in endothelial function during exacerbations of COPD may contribute to systemic vascular injury (32). Third, endothelial dysfunction may contribute to systemic vascular injury at a younger age or in those with as yet undefined subphenotypes of COPD. Finally, it is possible that a much larger study may find that a small proportion of the association between FEV_1 and atherosclerosis is mediated by endothelial dysfunction, although our results suggest that endothelial dysfunction is unlikley to be the dominant mechanism linking FEV_1 and atherosclerosis. For these reasons, endothelial dysfunction should continue to be actively investigated as a mechanism for atherosclerosis in those with lung disease, recognizing that the relationship among airflow limitation, endothelial dysfunction, and atherosclerosis is perhaps more complex than initially envisioned.

Nonetheless, there are multiple mechanisms besides endothelial dysfunction that may explain the association between airflow limitation and atherosclerosis. These have been the subject of multiple review articles and a detailed discussion is beyond the scope of this report (5, 33). Among these increased arterial stiffness and systemic inflammation are worthy of brief mention here. Increased arterial stiffness is associated with both emphysema and atherosclerosis; however, it remains unknown if it mediates the association between them (30). Systemic inflammation is a known contributor to atherosclerotic vascular injury (34), and a vigorous systemic inflammatory response accompanies airflow limitation (35). However, the pathogenesis of this inflammatory response and the specific components that may cause vascular injury remain elusive. Although endothelial dysfunction, systemic inflammation, and increased arterial stiffness are not mutually exclusive processes, one of them may be more directly responsible for the development of atherosclerosis in those with airflow limitation.

Our study has limitations. First, there were differences in methodology and inclusion criteria between the two cohorts. Specifically, prebronchodilator spirometry was performed in HeartSCORE; however, prebronchodilator and post-bronchodilator spirometry demonstrated the same associations with atherosclerosis and endothelial function in the SCCOR cohort. Also, not all the participants in the HeartSCORE study were smokers; however, they demonstrated the same findings as participants in the SCCOR study who were all smokers. Finally, we assessed coronary artery calcium using non-ECG-gated CT in SCCOR participants. However, this technique has been validated by multiple independent investigators (18–22), and results were

identical for coronary artery calcium assessed by ECG-gated cardiac CT (current gold standard) in HeartSCORE participants. Therefore, the methodology used in one cohort complemented that used in the other. Also, because findings were robust to these differences, we believe that reporting results from the two cohorts together was justified, and is a strength rather than a weakness of our study.

Second, it is difficult to determine the direction of the associations studied because analyses were cross-sectional. However, it is well accepted that airflow limitation and endothelial dysfunction are precursors rather than sequelae of atherosclerosis.

Third, it is possible that our sample size was inadequate to detect the statistical significance of a weak correlation between FEV_1 and endothelial function. However, it should be noted that each of the included cohorts is larger than any prior study on the topic, and had adequate power to detect the associations between airflow limitation and atherosclerosis, and endothelial dysfunction and atherosclerosis. The upper endpoint of the 95% CI for the correlation between FEV_1 and flow-mediated dilation in the SCCOR cohort ($r = 0.04$; n = 231; $P = 0.61$) was 0.16. Similarly, the upper endpoint of the 95% CI for the correlation between FEV_1 and reactive hyperemia index in the HeartSCORE cohort ($r = 0.09$; n = 328; $P = 0.13$) was 0.19. Therefore, we believe that the possibility of a true correlation between FEV_1 and endothelial function greater than 0.19 can reasonably be excluded among participants in the two cohorts.

Finally, our study did not include large numbers of participants with more severe lung disease, particularly in the HeartSCORE cohort, and the relationship between FEV_1, endothelial dysfunction, and atherosclerosis in those with moderate to severe lung disease should be further examined in future studies.

Our results suggest that endothelial dysfunction does not explain the association between airflow limitation and atherosclerosis. Instead, airflow limitation and endothelial dysfunction seem to be unrelated and mutually independent predictors of coronary and carotid atherosclerosis. These findings imply that the relationship between airflow limitation, endothelial dysfunction, and atherosclerosis is likely more complex than initially envisioned, and requires further examination in large unselected community-based cohorts. ■

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