

Comprehensive Assessment of Coronary Plaque Progression With Advanced Intravascular Imaging, Physiological Measures, and Wall Shear Stress: A Pilot Double-Blinded Randomized Controlled Clinical Trial of Nebivolol Versus Atenolol in Nonobstructive Coronary Artery Disease

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Background—We hypothesized that nebivolol, a β -blocker with nitric oxide-mediated activity, compared with atenolol, a β -blocker without such activity, would decrease oxidative stress and improve the effects of endothelial dysfunction and wall shear stress (WSS), thereby reducing atherosclerosis progression and vulnerability in patients with nonobstructive coronary artery disease.

Methods and Results—In this pilot double-blinded randomized controlled trial, 24 patients treated for 1 year with nebivolol 10 mg versus atenolol 100 mg plus standard medical therapy underwent baseline and follow-up coronary angiography with assessments of inflammatory and oxidative stress biomarkers, microvascular function, endothelial function, and virtual histology intravascular ultrasound. WSS was calculated from computational fluid dynamics. Virtual histology intravascular ultrasound segments were assessed for vessel volumetrics and remodeling. There was a trend toward more low-WSS segments in the nebivolol cohort ($P=0.06$). Low-WSS regions were associated with greater plaque progression ($P<0.0001$) and constrictive remodeling ($P=0.04$); conversely, high-WSS segments demonstrated plaque regression and excessive expansive remodeling. Nebivolol patients had decreased lumen and vessel areas along with increased plaque area, resulting in more constrictive remodeling ($P=0.002$). There were no significant differences in biomarker levels, microvascular function, endothelial function, or number of thin-capped fibroatheromas per vessel. Importantly, after adjusting for β -blocker, low-WSS segments remained significantly associated with lumen loss and plaque progression.

Conclusion—Nebivolol, compared with atenolol, was associated with greater plaque progression and constrictive remodeling, likely driven by more low-WSS segments in the nebivolol arm. Both β -blockers had similar effects on oxidative stress, microvascular function, and endothelial function.

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Key Words: coronary flow • coronary microvascular function • endothelial function • intravascular ultrasound • wall shear stress

Alterations in coronary wall shear stress (WSS) can interact with the endothelium to affect the distribution, progression, and pathophysiology of atherosclerosis,^{1–9} and

several studies have demonstrated the association between WSS magnitudes and plaque development.^{3–10} In particular, low WSS (<1.0 Pa) has been related to plaque

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An accompanying Video S1 is available at <http://jaha.ahajournals.org/content/5/1/e002764/suppl/DC1>

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progression,^{5–10} whereas high WSS (>2.5 Pa) has been associated with increased necrotic core area on virtual histology intravascular ultrasound (VH-IVUS), a marker of plaque vulnerability.^{3,4,9,10} The impact of valsartan and simvastatin on the proinflammatory effect of low WSS has been studied in the swine model¹¹; however, the effect of an endothelial protective pharmaceutical agent, such as nebivolol, on the interplay between WSS and the progression of human coronary atherosclerosis has not been investigated.

Nebivolol is a third-generation β_1 receptor–selective antagonist with nitric oxide–mediated vasodilatory effect approved by the US Food and Drug Administration for treatment of hypertension and in Europe for left ventricular systolic dysfunction. Previous studies have suggested that nebivolol decreases coronary microvascular resistance through an agonist effect on the endothelial β_3 receptor, which mediates nitric oxide– and endothelium-dependent vasorelaxation.^{12–16} Enhanced flow and nitric oxide availability may decrease vascular reactivity and lipid oxidation and prove beneficial in conditions such as atherosclerosis, in which oxidative stress leads to decreased endothelial nitric oxide synthase expression and nitric oxide bioavailability. Potential benefits from nebivolol therapy may be observed in measures of endothelial function, microvascular function, vascular remodeling, or epicardial plaque burden and composition.

Accordingly, we conducted a pilot double-blinded randomized controlled trial on the effect of 1 year of nebivolol versus atenolol therapy on the changes in the number of thin-capped fibroatheromas (TCFAs) per vessel and in coronary plaque volumetrics among different WSS categories in patients with nonobstructive coronary artery disease. We hypothesized that nebivolol, by decreasing oxidative stress and improving endothelial function, would favorably affect atherosclerosis progression compared with atenolol, a β -blocker without nitric oxide–mediated activity.

Methods

Participants and Study Design

This single-center double-blinded randomized trial (NCT01230892) enrolled patients with recurrent angina symptoms who presented to the cardiac catheterization laboratory at Emory University Hospital from 2010 to 2012 and were found to have coronary nonobstructive atherosclerotic lesions (<50% stenosis by angiography or <70% stenosis with fractional flow reserve >0.80). Patients provided written informed consent for participation in this study prior to the baseline cardiac catheterization and randomization.

Inclusion criteria included patients being on stable medical therapy, presentation of stable angina or non–ST-segment

elevation acute coronary syndrome, and coronary lesion in the proximal 60 mm of an epicardial vessel deemed significant enough by the operator to warrant further evaluation using physiology and VH-IVUS. Patients were excluded if they presented with cardiogenic shock, ejection fraction <30%, or significant hepatic, hematologic, or renal impairment; had a history of coronary artery bypass surgery or severe valvular heart disease; could not provide informed consent; or had any contraindication to β -blocker therapy. Anatomically, cases were excluded if there were lesions requiring revascularization or significant visual coronary collaterals.

After undergoing baseline coronary angiography with VH-IVUS and evaluation of coronary microvascular and endothelial function, patients were randomized (1:1) to nebivolol 10 mg daily (first week 5 mg daily) or atenolol 100 mg daily (first week 50 mg daily) for 1 year. Nebivolol 5 mg (Forest Laboratories) and atenolol 50 mg (AstraZeneca Pharmaceuticals) have equivalent efficacy for reducing blood pressure.¹⁷

The Investigational Drug Service at our institution performed simple randomization using the online program “Research Randomizer” to generate the randomization plan in blocks of 4; dispensed the blinded study medication (same tablet size, shape, and color, with the same bottles and labels); and maintained separate records using WebIDS, a fully Health Insurance Portability and Accountability Act–compliant system that maintains protocol and drug information, patient profiles, randomization assignments, inventory records, and dispensing and patient return information. Enrolled patients were treated for 12 \pm 3 months with the study drug plus standard medical therapy (aspirin, statin, and as needed sublingual nitroglycerin) followed by repeat coronary angiography with VH-IVUS and assessments of microvascular and endothelial function (Figure 1). Participants, care providers, and study investigators were blinded until after completion of the last follow-up visit.

The Emory University institutional review board approved the study, and there were no significant changes to the protocol after the trial commenced. The primary end point was the change in the number of TCFAs per vessel between baseline and follow-up. Secondary end points included inflammatory and oxidative stress biomarkers, endothelial function, microvascular function, static and serial arterial remodeling, and VH-IVUS plaque area and composition at baseline and 1-year follow-up.

Lipids and Biomarkers

Basic metabolic panel, complete blood count, fasting lipid panel, and C-reactive protein level were collected the day of angiography and measured by the hospital medical laboratory services. Samples for plasma glutathione and cystine measurements were collected immediately after obtaining vascu-

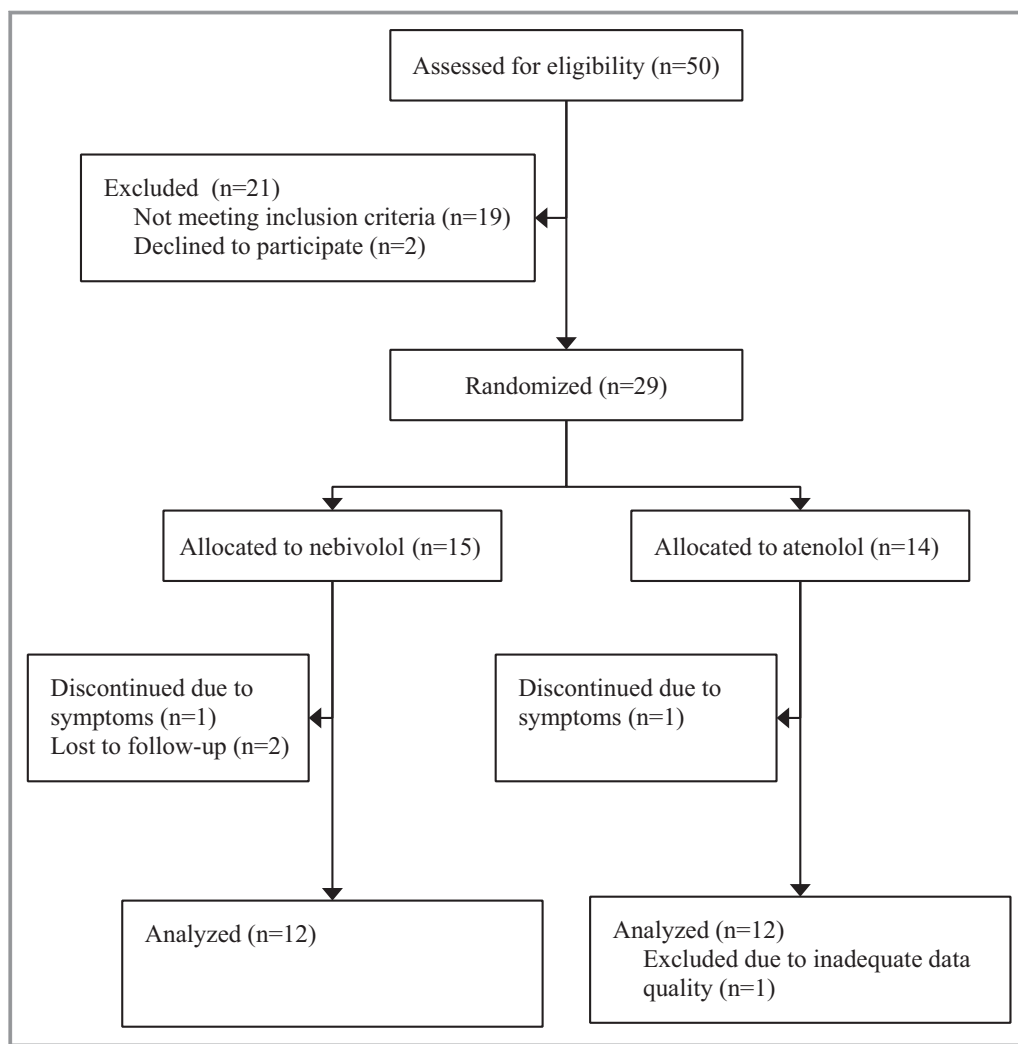


Figure 1. Study flowchart.

lar access in the cardiac catheterization laboratory, were frozen at -80°C , and were measured by the Emory University clinical biomarkers core laboratory. These samples have been shown to be stable under these conditions for 1 year.¹⁸

Cardiac Catheterization With Assessment of Endothelial and Microvascular Function

All β -blockers were withheld for ≥ 48 hours and long-acting nitrates were withheld for ≥ 24 hours prior to cardiac catheterization (both baseline and follow-up procedures). Procedures were performed in the morning after patients had fasted for at least 8 hours. Systolic and diastolic blood pressures and heart rate were measured prior to the start of the procedure. Patients underwent angiography in a biplane cardiac catheterization system (Toshiba America Medical Systems) using a standard 6F technique.

Pressure and velocity measurements were obtained using a 0.014-in pressure and Doppler flow velocity monitoring

guidewire (ComboWire: Volcano Corporation). For safety considerations, only the left coronary system was interrogated. The ComboWire was advanced to the guide catheter tip, at which the aortic pressure and guidewire pressures were equalized. Microvascular function was evaluated from pressure and velocity responses to intravenous adenosine infusion ($140 \mu\text{g}/\text{kg}$ per minute) for 3 minutes. At maximal hyperemia, fractional flow reserve was measured as the ratio of distal to aortic pressure, and hyperemic microvascular resistance was measured as the ratio of distal pressure to average peak flow velocity. Coronary flow velocity reserve was defined as the ratio of hyperemic to basal average peak flow velocity. Velocity measurements demonstrated good reproducibility, with a concordance correlation coefficient of 0.979 (95% CI 0.966–0.988).⁹

Endothelial function was assessed as the percentage change in coronary diameter and blood flow in response to intracoronary acetylcholine (off-label use) using Doppler flow velocity measurements and quantitative coronary angiography

(QAngio XA 7.3; Medis Medical Imaging Systems). Patients were first evaluated with 10^{-8} mol/L acetylcholine for safety prior to proceeding with 10^{-6} mol/L acetylcholine. Figure 2 demonstrates Doppler velocity envelopes prior to and during medication infusion in 1 patient at baseline and follow-up.

VH-IVUS Acquisition and Analysis

VH-IVUS acquisition was performed after administration of 200 μ g intracoronary nitroglycerin using phased-array 20 MHz Eagle Eye catheters and a s5 Imaging System (Volcano Corporation). Automated motorized pullback (0.5 mm/s) was performed, and VH-IVUS images were continuously acquired up to the guide catheter in the aorta (up to 60 mm of the proximal vessel). Angiography was used to record the catheter start position and its relationship to anatomic branching landmarks to aid with coregistration.

Offline analysis was performed at the Emory cardiovascular imaging and biomechanical core laboratory by experienced investigators who were blinded to the patients' clinical data according to the criteria of the American College of Cardiology Clinical Consensus document on IVUS,^{19,20} using VIAS

version 3.0 (Volcano Corporation) and echoPlaque 4.0.27 (INDEC Medical Systems). Measurements of the external elastic membrane (EEM), plaque, and lumen cross-sectional areas were performed for every recorded VH-IVUS cross-section (0.5-mm thickness), defined as a segment in the current analysis. Plaque burden was calculated as plaque area divided by EEM area. Segments involving bifurcating branch points or precluding complete lumen or vessel wall planimetry were excluded from analysis. Intraobserver analysis demonstrated good reproducibility for plaque area (concordance correlation coefficient 0.968 [95% CI 0.965–0.971]).⁹

Different nomenclatures have been used to describe arterial remodeling patterns. We defined static remodeling as the ratio of lesion to reference EEM area, also known as the remodeling index. We also used *excessive expansive*, *compensatory*, and *constrictive* to describe 3 patterns of serial remodeling.¹⁹ For each segment, positive Δ EEM area was defined as positive remodeling, and negative Δ EEM area was defined as constrictive remodeling. Segments with positive remodeling were further subdivided as compensatory if the ratio of Δ EEM area to Δ plaque area was between 0.0 and 1.0 or as excessive expansive otherwise.

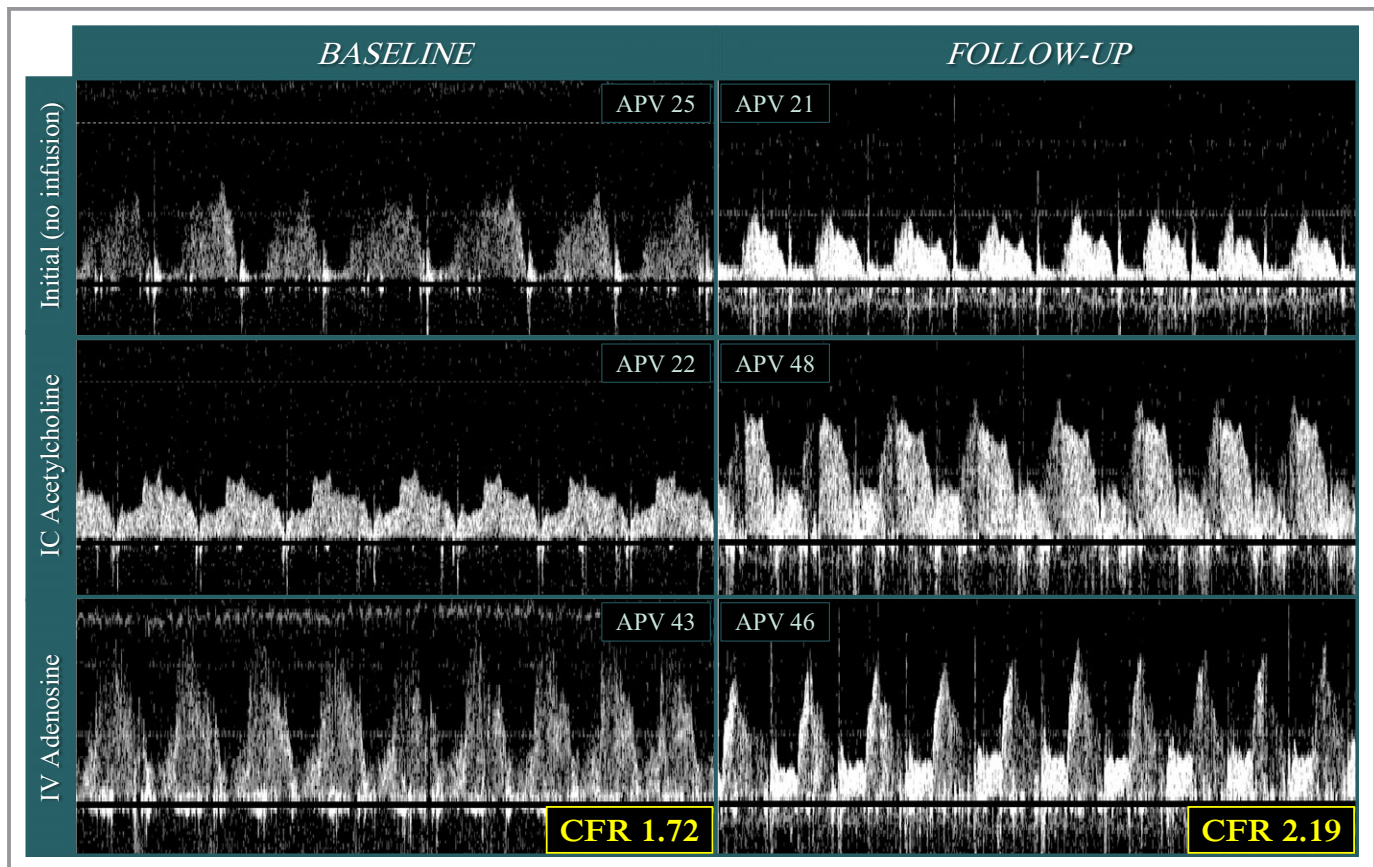


Figure 2. Doppler velocity waveforms obtained from a patient undergoing endothelial and microvascular function assessment at baseline and 1-year follow-up. APV indicates average peak velocity; CFR, coronary flow reserve; IC, intracoronary; IV, intravenous.

Finally, lesions were classified based on plaque composition as assessed by VH-IVUS (fibrous, fibrofatty, necrotic core, and dense calcium) for each segment.^{20,21} Fibroatheromas were defined as $\geq 40\%$ plaque burden and $\geq 10\%$ necrotic core; TCFAs were fibroatheromas with necrotic core abutting the lumen in at least 3 consecutive frames.

Computational Fluid Dynamics and Baseline WSS Analysis

WSS analysis methodology using the ANGUS method has been described previously.^{1,9} Briefly, the 3-dimensional path of the VH-IVUS catheter was determined using corresponding biplane angiographic projections acquired at the start of pullback. Each frame was rotated and aligned perpendicular to the catheter core to reconstruct the main artery of interest. Arterial branches were added based on information from angiography and VH-IVUS. Patient-specific pulsatile inlet boundary conditions were determined from Doppler wire-derived waveforms, and the outlets were assumed to be pressure-free. The reconstructed surface was meshed and imported into Fluent (ANSYS). After computing the pulsatile flow field in the region of interest, WSS was determined as a function of time in the cardiac cycle and spatial location around the lumen (Video S1, Figure 3) and then averaged over time and circumference at each cross-section for quantitative

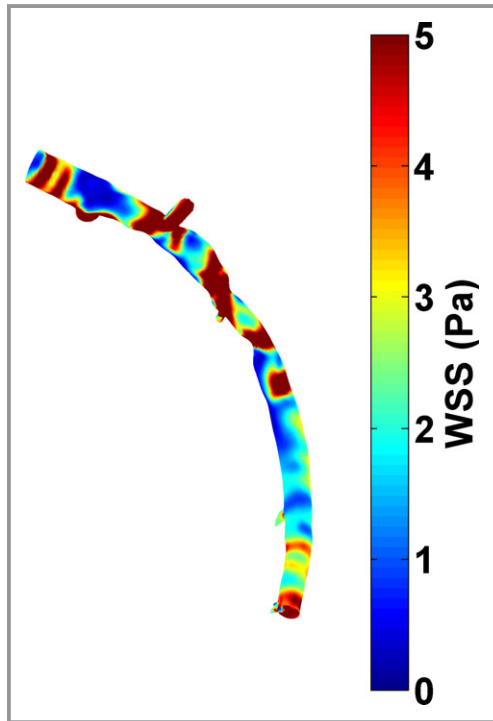


Figure 3. A patient-specific WSS profile of the left anterior descending coronary artery demonstrates areas of variable WSS magnitudes. WSS indicate wall shear stress.

analysis. Although WSS is a continuous and dynamic variable, based on previous cell culture and experimental and human data, WSS magnitudes were categorized as low (< 1.0 Pa), intermediate (≥ 1.0 and < 2.5 Pa), or high (≥ 2.5 Pa).^{3,9,22,23}

Statistical Analysis

Sample size was not determined strictly from effect sizes because the purpose of this pilot study was to generate data to power future clinical trials. It was estimated using previous comparative studies between nebivolol and atenolol on endothelial and microvascular function assuming a 0.05 significance level and 0.80 power value.^{13,15,16}

Continuous variables are described as mean \pm SD or median and interquartile range, as appropriate, and categorical variables are described as counts and proportions. For patient-level analyses, the chi-square statistic was used to compare categorical variables (eg, hypertension), and the 2-sample *t* test was used for continuous variables (eg, age). The differences between baseline and follow-up patient-level measures (eg, systolic blood pressure) were computed for each group, and the differences between the 2 groups were compared with the 2-sample *t* test.

Correlated error is introduced by the clustering of arterial segments within patients. To correct for this, *P* values were adjusted with linear regression for continuous outcomes (eg, Δ lumen area) and ordered logistic regression for categorical variables (eg, serial arterial remodeling). In both situations, the Huber White Sandwich Estimator was used to adjust the *P* values for correlated error.^{24,25} To further examine the association of WSS, drug, and change measures, mixed models with WSS and drug as predictor variables were run. Although there are many observations at the VH-IVUS segmental level, the effective sample size is 24 participants, and 2 covariates were determined to be a reasonable limit. Analyses were performed using SAS 9.3 (SAS Institute) or Stata 13.1 (StataCorp LP). $P < 0.05$ was established as the level of statistical significance.

Results

Study Participants, Entire Cohort

Fifty patients consented to study participation prior to baseline coronary angiography. Two patients who underwent initial cardiac catheterization developed severe vasospasm during 10^{-6} mol/L acetylcholine infusion. They were successfully treated during the procedure and did well clinically but did not participate further in this trial or undergo randomization. Another 19 patients did not meet angiographic inclusion criteria and were considered screen failures. Consequently, 29 patients were enrolled in the study and underwent

randomization. A total of 25 patients returned for follow-up; 1 case was excluded from analysis due to poor data quality (Figure 1).

For the remaining 24 patients, physiological interrogation was performed in the left circumflex artery for 1 patient and in the left anterior descending artery for 23 patients. Table 1 displays baseline patient characteristics. At follow-up, both

Table 1. Patient Demographics and Characteristics

	Total (n=24)	Atenolol (n=12)	Nebivolol (n=12)	P Value
Age, y	52.5±10.3	49.8±10.7	55.2±9.5	0.21
Male	9 (38)	5 (42)	4 (33)	0.67
White race	18 (75)	8 (67)	10 (83)	0.35
Body mass index	29.1±5.8	30.0±6.4	28.2±5.2	0.46
Follow-up time period, months	12.4±1.0	12.5±0.6	12.4±1.3	0.81
Cardiovascular risk factors				
Hypertension	16 (67)	9 (75)	7 (58)	0.39
Diabetes mellitus	4 (17)	2 (17)	2 (17)	1.00
Dyslipidemia	18 (75)	8 (67)	10 (83)	0.35
Prior myocardial infarction	4 (17)	2 (17)	2 (17)	1.00
Smoking history	12 (50)	7 (58)	5 (42)	0.41
Family history of coronary disease	11 (46)	4 (33)	7 (58)	0.22
Presentation				
Non-ST-segment elevation myocardial infarction	1 (4)	1 (8)	0 (0)	
Unstable angina	5 (21)	2 (17)	3 (25)	0.52
Stable angina	17 (71)	8 (67)	9 (75)	
Medication use				
Aspirin	20 (83)	10 (83)	10 (83)	1.00
P2Y ₁₂ inhibitor	6 (25)	3 (25)	3 (25)	1.00
Statin	23 (96)	12 (100)	11 (92)	0.31
Calcium channel blocker	11 (46)	7 (58)	4 (33)	0.22
Long-acting nitrate	17 (71)	10 (83)	7 (58)	0.18
ACE inhibitor or ARB	6 (25)	3 (25)	3 (25)	1.00

Values are expressed as n (%) or mean±SD. ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

systolic and diastolic blood pressures decreased numerically (*P* value not significant), but heart rate changed significantly by −5 beats per minute (interquartile range −11 to +1 beats per minute; *P*=0.02). Low-density lipoprotein cholesterol level also increased by 10 mg/dL (interquartile range −3 to +29 mg/dL; *P*=0.04). There were no significant changes in coronary physiology or endothelial function between baseline and follow-up (Table 2).

Baseline mean VH-IVUS run length was 58±17 mm, lumen area was 11.84±5.38 mm², EEM area was 17.08±7.35 mm², and plaque area was 5.24±4.07 mm². At follow-up, the change in EEM area was −0.33±2.31 mm² (*P*<0.0001), in lumen area was −0.51±2.54 mm² (*P*<0.0001), in plaque area was 0.18±2.21 mm² (*P*<0.0001), in fibrous area was −0.01±1.33 mm² (*P*=0.67), in fibrofatty area was −0.01±0.38 mm² (*P*=0.22), in necrotic core area was −0.02±0.64 mm² (*P*=0.07), and in dense calcium area was 0.03±0.21 mm² (*P*<0.0001).

Relationship Between Baseline Coronary WSS, Serial Arterial Remodeling, and Plaque Progression

Coronary WSS was calculated in 1843 VH-IVUS segments using computational fluid dynamics modeling. There were 428 (23%) low-, 920 (50%) intermediate-, and 495 (27%) high-WSS segments. At follow-up, low-WSS segments demonstrated an increase in plaque area (*P*<0.0001) and a decrease in lumen area (*P*<0.0001) compared with intermediate- and high-WSS segments (Table 3) and more constrictive remodeling (*P*=0.04) (Figure 4).

Comparisons Between Nebivolol and Atenolol

Between the 2 cohorts, there were no significant differences in clinical characteristics or cardiac medications (Table 1) or other baseline and follow-up variables such as cholesterol levels, inflammatory and oxidative stress biomarkers, coronary flow velocity reserve, hyperemic microvascular resistance, or coronary endothelial function (Table 2). There was a trend of more segments in the nebivolol cohort with low WSS (317 versus 111) and fewer segments with high WSS (175 versus 320) compared with the atenolol arm (Figure 5).

On VH-IVUS, nebivolol segments demonstrated significantly decreased EEM area (*P*=0.02) and lumen area (*P*=0.004) but similar changes in plaque components and number of TCFA per vessel compared with atenolol (Table 2). Nebivolol also showed increased plaque area at follow-up (*P*<0.001), whereas atenolol demonstrated plaque regression (*P*=0.047); however, the difference between the 2 arms was not significant (*P*=0.27). With respect to arterial remodeling, static remodeling (remodeling index) was similar between the

Table 2. Patient and Vessel Characteristics at Baseline and Change After 1 Year

	Baseline			Change After 1 Year		
	Atenolol (n=12)	Nebivolol (n=12)	P Value	Atenolol (n=12)	Nebivolol (n=12)	P Value
Vital signs						
Systolic BP, mm Hg	137 [122, 145]	143 [122, 159]	0.51	−8 [−25, 10]	−7 [−20, 1]	0.95
Diastolic BP, mm Hg	73 [71, 85]	78 [69, 92]	0.63	0 [−8, 14]	−8 [−14, 6]	0.08
Heart rate, bpm	71 [60, 85]	68 [62, 78]	0.91	−5 [−11, 6]	−6 [−11, −1]*	0.66
Fasting lipid panel						
Total cholesterol, mg/dL	158 [136, 171]	149 [119, 160]	0.13	15 [−1, 31]	14 [−2, 55]	0.80
HDL cholesterol, mg/dL	48 [38, 54]	44 [43, 54]	0.75	3 [−5, 16]	−3 [−8, 2]	0.16
LDL cholesterol, mg/dL	87 [69, 104]	81 [72, 115]	0.52	6 [−3, 29]	13 [−4, 35]	0.89
Markers of inflammation and oxidative stress						
C-reactive protein, mg/L	1.87 [0.50, 3.82]	2.05 [1.30, 4.06]	0.48	0.33 [−1.72, 2.48]	0.07 [−0.78, 0.42]	0.72
Cystine, μmol/L	84 [72, 112]	88 [79, 108]	0.41	10 [−4, 20]	17 [−7, 43]	0.37
Glutathione, μmol/L	1.04 [0.87, 1.60]	0.98 [0.79, 1.26]	0.74	−0.13 [−0.56, 0.46]	−0.01 [−0.04, 0.17]	0.52
Cystine/glutathione ratio	84 [63, 100]	88 [69, 139]	0.53	31 [−2, 44]	15 [−24, 48]	0.78
Physiology, endothelial function, and vessel characteristics						
FFR	0.98 [0.94, 1.00]	0.91 [0.90, 0.98]	0.08	−0.02 [−0.08, 0.01]	−0.01 [−0.03, 0.05]	0.39
CFR	2.07 [1.82, 2.38]	1.87 [1.62, 2.33]	0.74	0.41 [0.06, 0.48]	0.71 [−0.58, 1.26]	0.97
HMR	1.75 [1.47, 2.66]	1.91 [1.46, 2.21]	0.89	0.07 [−0.54, 1.07]	−0.06 [−0.24, 0.43]	1.00
%Diameter change to ACh	−5 [−22, 3]	−2 [−8, 15]	0.47	2 [−11, 30]	3 [−12, 25]	0.89
%CBF change to ACh	60 [−11, 225]	142 [37, 181]	0.81	−5 [−172, 121]	87 [30, 385]	0.47
TCFAs per patient	2.2±1.2	1.6±1.0	0.27	−0.4±0.8	−0.3±1.0	0.94
Segment characteristics						
VH-IVUS run length, mm	54.3±10.9	62.5±21.1	0.25			
Minimum lumen area, mm ²	6.11 [3.85, 7.15]	4.15 [3.87, 7.77]	0.93			
Static remodeling	0.60±0.19	0.58±0.22	0.50			
EEM area, mm ²	16.5±7.3	17.5±7.3	0.76	0.20±1.99*	−0.78±2.46*	0.02
Lumen area, mm ²	10.9±4.5	12.7±5.9	0.41	0.33±2.08*	−1.22±2.68*	0.004
Plaque area, mm ²	5.7±4.8	4.9±3.3	0.66	−0.13±2.44*	0.44±1.95*	0.27
FI area, mm ²	1.67±2.86	1.08±1.66	0.51	−0.16±1.58*	0.14±1.02*	0.26
FF area, mm ²	0.23±0.46	0.16±0.35	0.57	−0.04±0.38*	0.02±0.38*	0.27
NC area, mm ²	0.48±1.07	0.36±0.80	0.77	−0.07±0.76*	0.03±0.49*	0.35
DC area, mm ²	0.09±0.17	0.15±0.38	0.28	0.01±0.17*	0.04±0.24*	0.34

Values are expressed as mean±SD or median [IQR]. ACh indicates acetylcholine; BP, blood pressure; bpm, beats per minute; CBF, coronary blood flow; CFR, coronary flow reserve; DC indicates dense calcium; EEM, external elastic membrane; FF, fibrofatty; FFR, fractional flow reserve; FI, fibrous; HDL, high-density lipoprotein; HMR, hyperemic myocardial resistance; LDL, low-density lipoprotein; NC, necrotic core; RI, remodeling index; TCFAs, thin-capped fibroatheromas; VH-IVUS, virtual histology intravascular ultrasound.

*Significant change from baseline ($P<0.05$).

2 groups, but serial remodeling was significantly different ($P=0.002$), with nebivolol segments demonstrating more constrictive remodeling (73% versus 46%) and less excessive expansive remodeling (16% versus 39%) than atenolol (Figure 6).

These observations could have been related to differences in baseline WSS patterns between the arms or to pharmaco-

logical therapy with the 2 different β -blockers, so VH-IVUS results were stratified by categories of WSS (Table 4) and β -blocker (Table 5). Among low-WSS segments (Table 4), those treated with nebivolol demonstrated decreased Δ EEM area compared with atenolol ($P=0.02$), consistent with a higher percentage of constrictive serial remodeling (83% versus 63%).

Table 3. Comparison of the Change in Virtual Histology Intravascular Ultrasound Measures After 1 year by Baseline WSS Category

Characteristic	Low WSS (n=428)	Intermediate WSS (n=920)	High WSS (n=495)	P Value
ΔLumen area, mm ²	-1.17±1.24	-0.64±1.61	0.61±2.15	<0.0001
ΔEEM area, mm ²	-0.70±1.32	-0.37±1.29	0.05±1.82	0.40
ΔPlaque area, mm ²	0.47±1.37	0.27±1.82	-0.56±2.77	<0.0001
ΔFibrous area, mm ²	0.21±0.76	0.02±1.08	-0.47±1.64	<0.0001
ΔFibrofatty area, mm ²	0.04±0.30	-0.01±0.24	-0.05±0.35	<0.0001
ΔNecrotic core area, mm ²	-0.07±0.71	-0.01±0.66	-0.17±0.88	<0.0001
ΔDense calcium area, mm ²	-0.01±0.22	0.04±0.22	0.04±0.26	<0.0001

Values are expressed as mean±SD. EEM indicates external elastic membrane; WSS, wall shear stress.

Table 5 compares VH-IVUS changes by WSS categories within each β-blocker cohort. Compared with high-WSS segments, low-WSS segments demonstrated greater decrease in lumen area ($P<0.0001$) and increase in plaque area ($P<0.01$). After adjustment for β-blocker, WSS category remained an independent predictor of changes in lumen area ($P<0.0001$) and plaque area ($P<0.0001$).

Discussion

This investigation is the first randomized controlled clinical trial studying the impact of a β₁-blocker with endothelial β₃-receptor agonist activity on the proatherosclerotic effect of coronary WSS. The major observations were as follows: First, there was greater plaque progression ($P<0.0001$) and constrictive remodeling in segments with low WSS ($P=0.04$) and, conversely, greater plaque regression and excessive expansive remodeling in segments with high WSS. Second,

there were greater reductions in lumen area ($P=0.004$) and vessel area ($P=0.02$), resulting in more constrictive remodeling, with nebivolol ($P=0.002$), likely driven by a trend toward more low-WSS segments in the nebivolol cohort ($P=0.06$). Third, after adjusting for the type of β-blocker therapy, low-WSS segments remained significantly associated with lumen loss and plaque progression ($P<0.0001$). Fourth, there were no significant differences in oxidative stress or inflammatory biomarker levels, endothelial function, microvascular function, or number of TCFA per vessel between nebivolol and atenolol.

Coronary atherosclerosis pathophysiology operates through complex multitude of pathways, including endothelial dysfunction as a conduit for systemic risk factors to affect the vasculature.^{18,26,27} The considerable number of investigations into the effects of WSS magnitudes on plaque development emphasizes this complexity. Low WSS can promote atherogenesis by changing endothelial cell morphology,² enhancing

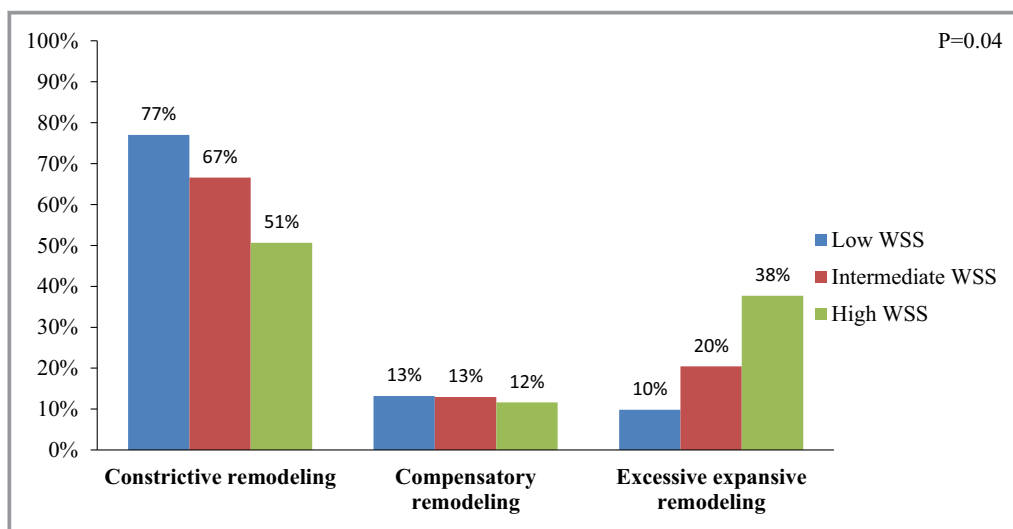


Figure 4. Serial arterial remodeling by baseline WSS categories at 1 year follow up. P value is for comparison of frequency of 3 remodeling groups across 3 WSS categories. WSS indicate wall shear stress.

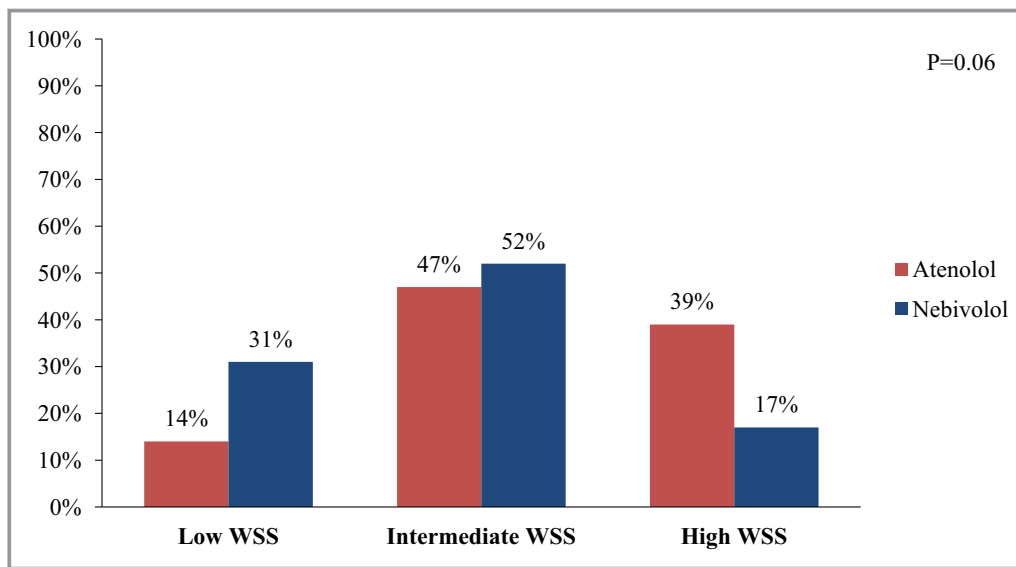


Figure 5. Distribution of baseline WSS by β -blocker. *P* value is for comparison of the frequency of 3 WSS categories across 2 β -blockers. WSS indicates wall shear stress.

production of reactive oxygen species and inflammatory molecules,²² and stimulating vascular smooth muscle cell migration.^{8,28} In our study, we observed that low-WSS regions were associated with more constrictive remodeling ($\Delta\text{EEM} -0.70 \text{ mm}^2$, $P=0.04$) and an absolute increase in plaque area of 0.47 mm^2 ($P<0.0001$). Conversely, high WSS can also contribute to plaque destabilization.^{4,10,29–31} Histological studies have implicated high WSS in smooth muscle cell apoptosis and proteoglycan matrix degradation,²⁹ whereas animal studies have demonstrated increased mechanical strain, vascular inflammation, macrophage activity, and expansive remodeling in high-WSS regions.^{30,32} We previously observed in human coronary arteries that, compared with intermediate-WSS segments, high-WSS segments trended toward more excessive expansive arterial remodeling,^{9,23} an exaggerated remodeling pattern in which both vessel and lumen dimensions increase proportionally more than plaque area. Although this type of remodeling in high-WSS segments may be partially explained by adaptive arterial enlargement in an attempt to restore WSS to a more physiological level,³¹ it can also drive plaque progression through continued lipid accumulation and inflammation. In this study, we demonstrated again that high-WSS regions were compensated by excessive expansive remodeling (Figure 5) with fibrous and fibrofatty tissue regression (Table 3).

The observation that nebivolol patients had more constrictive remodeling and lumen loss compared with atenolol patients in this study was interesting. Although this was a double-blinded randomized controlled trial, the nebivolol cohort had a numerically greater number of low coronary WSS segments per patient compared with the atenolol cohort (26.4 versus 9.3, $P=0.06$), despite having similar baseline

plaque areas (4.9 mm^2 versus 5.7 mm^2 , $P=0.66$), static vascular remodeling (remodeling index 0.58 versus 0.60, $P=0.50$), and coronary flow reserve (1.87 versus 2.07, $P=0.74$). After adjusting for type of β -blocker, low WSS remained significantly associated with plaque progression and lumen loss (both $P<0.0001$), suggesting that WSS and not type of β -blocker therapy was driving these findings.

Interestingly, in our study, nebivolol and atenolol had similar changes in lipid profiles, inflammation and oxidative stress biomarkers, and coronary endothelial and microvascular function. In another study of hypertensive patients, we also observed no differences between nebivolol and metoprolol regarding pulse wave velocity, a noninvasive measurement of arterial stiffness and endothelial function, or oxidative stress biomarker levels.³³ In addition, there were no significant differences between nebivolol and atenolol with respect to necrotic core area or the number of TCFA per vessel. Taken together, the current investigation demonstrates no significant favorable or adverse effects of nebivolol compared with atenolol on comprehensive atherosclerosis phenotyping.

Despite the multidimensional nature of atherosclerosis pathophysiology, previous mechanistic investigations into the impact of pharmacotherapies on atherosclerosis development have generally relied on basic measures of plaque assessment such as the change in carotid intima media thickness^{34,35} or coronary atheroma volume.^{36–38} These measures may underestimate the effect of antiatherosclerotic therapies on the multiple known atherosclerotic pathways and processes. To our knowledge, no prior investigation has comprehensively examined the incremental effect of a potential antiatherosclerotic therapy on epicardial and microvascular endothelial-dependent and -independent function, atherosclerosis burden and com-

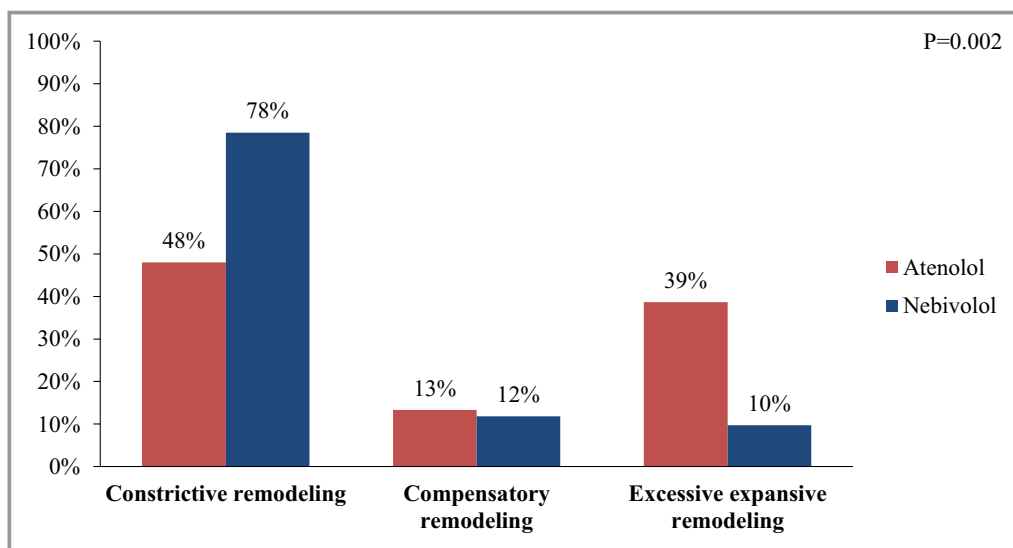


Figure 6. Serial arterial remodeling by β -blocker at 1-year follow-up. *P* value is for comparison of the frequency of 3 remodeling groups across 2 β -blocker categories.

position, static and serial arterial remodeling, and oxidative stress and inflammatory pathways in the context of coronary WSS. This investigation offers a blueprint for how novel cardiovascular therapies can be evaluated, given the numerous possible mechanisms for therapeutic intervention.

Limitations

The study was powered for VH-IVUS end points but may not have been adequately powered to detect changes in endothelial and microvascular function or number of TCFA per artery. Although the nebivolol dose used in this trial was approved for clinical use, the effective concentration may be too low or the

duration of follow-up insufficient to affect the prespecified end points. Nevertheless, this study is the most comprehensive atherosclerosis phenotyping double-blinded randomized controlled trial performed thus far and can establish the methodology for future coronary atherosclerosis trials for novel pharmaceutical agents such as protein convertase subtilisin/kexin type 9 inhibitors.

Plaque composition data are derived from VH-IVUS, which has inherent limitations compared with histology but can be performed in vivo in coronary arteries and has been validated by several studies.^{6–9,20,21} In addition, although there were many arterial segments, the effective sample size was 24 participants; this limits the number of covariates used in

Table 4. Comparison of Change in Virtual Histology Intravascular Ultrasound Measures by β -Blocker, Stratified by Baseline Wall Shear Stress Categories

	Low WSS			Intermediate WSS			High WSS		
	Atenolol (n=111)	Nebivolol (n=317)	<i>P</i> Value	Atenolol (n=382)	Nebivolol (n=538)	<i>P</i> Value	Atenolol (n=320)	Nebivolol (n=175)	<i>P</i> Value
Δ Lumen area, mm ²	-0.80±1.15	-1.29±1.25	0.22	0.21±1.79	-1.24±1.15	0.002	1.15±2.38	-0.39±1.08	0.02
Δ EEM area, mm ²	0.01±1.06	-0.95±1.31	0.02	-0.02±1.53	-0.62±1.02	0.15	0.29±1.96	-0.40±1.44	0.43
Δ Plaque area, mm ²	0.81±1.05	0.35±1.45	0.30	-0.23±2.38	0.62±1.15	0.28	-0.86±3.23	-0.01±1.45	0.30
Δ FI area, mm ²	0.36±0.84	0.16±0.72	0.23	-0.18±1.44	0.16±0.68	0.40	-0.66±1.94	-0.13±0.79	0.31
Δ FF area, mm ²	0.04±0.23	0.03±0.32	0.47	-0.03±0.25	0.00±0.24	0.77	-0.09±0.38	0.01±0.27	0.34
Δ NC area, mm ²	0.07±0.34	-0.12±0.79	0.45	-0.15±0.92	0.09±0.35	0.14	-0.25±1.06	-0.01±0.30	0.25
Δ DC area, mm ²	-0.01±0.12	-0.01±0.24	0.78	0.01±0.16	0.06±0.25	0.19	0.02±0.22	0.09±0.31	0.15

P-values are for comparison between 2 β -blocker categories. DC indicates dense calcium; EEM, external elastic membrane; FF, fibrofatty; FI, fibrous; NC, necrotic core; WSS, wall shear stress.

Table 5. Comparison of Change in VH-IVUS Measures by Baseline WSS Categories, Stratified by Type of β -Blocker

	Atenolol				Nebivolol			
	Low WSS (n=111)	Intermediate WSS (n=382)	High WSS (n=320)	P Value	Low WSS (n=317)	Intermediate WSS (n=538)	High WSS (n=175)	P Value
Δ Lumen area, mm ²	-0.80±1.15	0.21±1.79	1.15±2.38	<0.0001	-1.29±1.25	-1.24±1.15	-0.39±1.08	<0.0001
Δ Vessel area, mm ²	0.01±1.06	-0.02±1.53	0.29±1.96	0.002	-0.95±1.31	-0.62±1.02	-0.40±1.44	<0.0001
Δ Plaque area, mm ²	0.81±1.05	-0.23±2.38	-0.86±3.23	<0.0001	0.35±1.45	0.62±1.15	-0.01±1.45	0.006
Δ FI area, mm ²	0.36±0.84	-0.18±1.44	-0.66±1.94	<0.0001	0.16±0.72	0.16±0.68	-0.13±0.79	<0.0001
Δ FF area, mm ²	0.04±0.23	-0.03±0.25	-0.09±0.38	<0.0001	0.03±0.32	0.00±0.24	0.01±0.27	0.02
Δ NC area, mm ²	0.07±0.34	-0.15±0.92	-0.25±1.06	<0.0001	-0.12±0.79	0.09±0.35	-0.01±0.30	0.01
Δ DC area, mm ²	-0.01±0.12	0.01±0.16	0.02±0.22	0.57	-0.01±0.24	0.06±0.25	0.09±0.31	<0.0001

P-values are for comparison among 3 WSS categories. DC indicates dense calcium; FF, fibro-fatty; FI, fibrous; NC, necrotic core; WSS, wall shear stress.

multivariate analysis. Finally, this was a randomized clinical trial between nebivolol and atenolol; however, the constrictive remodeling seen in the nebivolol arm is likely related to the greater number of baseline segments with low WSS. Although randomization can be readily performed with prespecified balance in demographics, it is currently impractical, given the time- and resource-intensive nature of computational fluid dynamic modeling, to randomize patients based on their distribution of coronary WSS segments.

Conclusions

Compared with atenolol, nebivolol demonstrated greater luminal reduction and constrictive remodeling, but this was likely driven by the higher number of low-WSS segments in the nebivolol cohort. There were no significant differences in coronary endothelial function or microvascular function between treatment groups. This study suggests that any impact of an endothelial β_3 -receptor agonist on the effects of WSS magnitudes or on the natural history of epicardial and microvascular atherosclerosis, if present, is minimal. Importantly, it lays down the methodological framework for future mechanistic evaluation of pharmacotherapy on human coronary atherosclerosis.

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