Enhanced External Counterpulsation Improves Peripheral Artery Flow-Mediated Dilation in Patients With Chronic Angina

A Randomized Sham-Controlled Study

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Background—Mechanisms responsible for anti-ischemic benefits of enhanced external counterpulsation (EECP) remain unknown. This was the first randomized sham-controlled study to investigate the extracardiac effects of EECP on peripheral artery flow-mediated dilation.

Methods and Results—Forty-two symptomatic patients with coronary artery disease were randomized (2:1 ratio) to thirty-five 1-hour sessions of either EECP (n=28) or sham EECP (n=14). Flow-mediated dilation of the brachial and femoral arteries was performed with the use of ultrasound. Plasma levels of nitrate and nitrite, 6-keto-prostaglandin F1α, endothelin-1, asymmetrical dimethylarginine, tumor necrosis factor-α, monocyte chemoattractant protein-1, soluble vascular cell adhesion molecule, high-sensitivity C-reactive protein, and 8-isoprostane were measured. EECP increased brachial (+51% versus +2%) and femoral (+30% versus +3%) artery flow-mediated dilation, the nitric oxide turnover/production markers nitrate and nitrite (+36% versus +2%), and 6-keto-prostaglandin F1α (+71% versus +1%), whereas it decreased endothelin-1 (-25% versus +5%) and the nitric oxide synthase inhibitor asymmetrical dimethylarginine (−28% versus +0.2%) in treatment versus sham groups, respectively (all P<0.05). EECP decreased the proinflammatory cytokines tumor necrosis factor-α (−16% versus +12%), monocyte chemoattractant protein-1 (−13% versus +0.2%), soluble vascular cell adhesion molecule-1 (−6% versus +1%), high-sensitivity C-reactive protein (−32% versus +5%), and the lipid peroxidation marker 8-isoprostane (−21% versus +1.3%) in treatment versus sham groups, respectively (all P<0.05). EECP reduced angina classification (−62% versus 0%; P<0.001) in treatment versus sham groups, respectively.

Conclusions—Our findings provide novel mechanistic evidence that EECP has a beneficial effect on peripheral artery flow-mediated dilation and endothelial-derived vasoactive agents in patients with symptomatic coronary artery disease. (Circulation. 2010;122:1612-1620.)

Key Words: angina ■ endothelin ■ inflammation ■ nitric oxide ■ vasodilation

Enhanced external counterpulsation (EECP) is a noninvasive outpatient therapy for patients with chronic stable angina who fail to respond to standard revascularization procedures and aggressive pharmacotherapy. Data from the International Patient Registry demonstrate that EECP effectively decreases the frequency of anginal episodes and nitrate usage and increases exercise tolerance.1-3 However, the mechanism(s) responsible for the salutary clinical benefits of EECP remains largely unknown. This was the first randomized sham-controlled study to investigate the extracardiac effects of EECP on peripheral artery flow-mediated dilation.

Clinical Perspective on p 1620

The central hypothesis, in most investigations conducted to elucidate the mechanism of action, is that EECP may promote coronary angiogenesis. However, this understanding of EECP is a theory and remains unconfirmed in randomized clinical trials.3,4 In an international trial (7 centers; 175 chronic stable angina patients), EECP failed to elicit improved cardiac perfusion in 46% of study subjects.5 In a recent US trial (6 US university hospitals; 37 patients with stable chronic angina), EECP failed to improve myocardial blood flow to and within ischemic regions of the myocardium.6 However, despite negligible improvements in myocardial perfusion, ≈85% of patients in EECP clinical trials experience reduction in angina.1,5 To date, possible alternative extracardiac mechanisms associated with EECP have received little attention.7
Therefore, the purpose of the present study was to conduct the first randomized sham-controlled investigation to assess the extracardiac effects of EECP on peripheral artery flow-mediated dilation (FMD) and endothelial-derived vasoactive agents in patients with refractory angina. Endothelial dysfunction is a systemic process and not necessarily confined to vascular beds with clinically overt atherosclerosis. 8 We hypothesized that EECP would improve FMD in peripheral muscular conduit arteries. We further hypothesized that EECP would elicit commensurate changes in endothelial-derived vasoactive agents, plasma inflammatory cytokines, and indices of oxidative damage. We reasoned that arterial FMD and the balance of endothelial-derived vasoactive agents could be improved by enhanced hemodynamic shear stress with each inflation/deflation cycle of the compressive cuffs. 9

Methods

Baseline Status of Subjects

Forty-two consecutive patients with chronic stable angina referred for EECP treatment were randomized in a 2:1 manner into either an EECP treatment group or a sham EECP control group. The patients were enrolled for EECP treatment between November 2004 and June 2009 because they experienced chronic angina for >3 months caused by myocardial ischemia in the presence of angiographic multivessel coronary artery disease (CAD) that could not be controlled by a combination of medical therapy, angioplasty/stent, and/or coronary artery bypass graft (CABG) surgery. The study was approved by the institutional review board of the University of Florida, and written informed consent was obtained from all patients.

Exclusion Criteria

Exclusion criteria were absence of ST-segment depression (1 mm minimum) during exercise testing, >75 years of age, CABG within the past 3 months or percutaneous coronary intervention in the past 6 months, cardiac catheterization for any reason within the past 2 weeks, arrhythmia that would significantly interfere with triggering of the EECP device, symptomatic heart failure and/or left ventricular ejection fraction <30%, valvular heart disease, implantable cardioverter-defibrillator if triggered within the past 6 months, history of deep vein thrombosis, uncontrolled hypertension, pregnancy, pulmonary congestion, and systemic hypotension.

EECP and Sham Groups

Patients in the EECP (n = 28) and sham (n = 14) groups received thirty-five 1-hour daily sessions of EECP for 7 consecutive weeks with cuff inflation pressures of 300 and 70 mm Hg, respectively. It was determined previously that 70 mm Hg inflation pressure for sham is adequate to preserve the appearance and feel of EECP application but insufficient to alter blood pressure. 1 EECP equipment (Vasomedical, Westbury, NY) has been described previously. 1 Blood Collection and Biochemical Assays

Venipuncture was performed before and after 35 sessions of EECP or sham. Plasma levels of tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and high-sensitivity C-reactive protein (hsCRP) were determined by enzyme-linked immunosorbent assay (Quantikine; R&D Systems Inc, Minneapolis, Minn). The intra-assay and interassay coefficients of variation were 4.6% and 7.7% for TNF-α, 4.2% and 6.9% for MCP-1, 3.4% and 6.1% for sVCAM-1, and 4.6% and 8.0% for hsCRP, respectively. Serum lipids and glucose were measured in hospital laboratories by validated techniques.

Statistical Analysis

All statistical analyses were performed with the use of SPSS version 18.0 for Windows (SPSS, Chicago, Ill). Continuous variable data are presented as mean ± SD. All data were tested for normal distribution with the Shapiro-Wilk test for normality. An α level of P < 0.05 was required for statistical significance. Repeated-measures ANOVA was used to evaluate the continuous primary dependent variables brachial and femoral artery FMD, the secondary dependent variables NOx and ET-1, the plasma biomarkers, patient characteristics, and all other data. When a statistically significant main effect between treatment and sham groups was determined, within-group repeated-measures ANOVAs were used to determine which specific groups differed.

Peripheral FMD

The FMD technique was used to determine endothelial-dependent reactivity in the brachial and femoral arteries before and after 35 sessions of EECP or sham. Brachial artery FMD was assessed in the right arm with the use of a high-resolution ultrasound machine (ATL HDI 3000; Advanced Technologies Laboratories, Bothell, Wash) equipped with a 10.5-MHz transducer. 11 Briefly, resting baseline end-diastolic brachial diameters and blood velocity were obtained with the transducer placed 3 to 5 cm above the antecubital fossa. Reactive hyperemia was produced by inflating a blood pressure cuff placed on the upper forearm for 5 minutes at 200 mm Hg, followed by a rapid deflation. The brachial artery was imaged and recorded for 3 minutes after cuff deflation. Ultrasound images were recorded on a super VHS videocassette for offline electronic image analysis with the use of Image Pro Software (Image Pro Data Translation Inc, Marlboro, Mass). Brachial artery diameters were determined during end-diastole (gated with ECG R wave) by measuring the distance between the near and far walls of the intima. Peak brachial FMD was expressed as a percent increase from baseline (FMD%). FMD% is influenced by baseline diameter, and therefore absolute changes (Δmm) in diameter were also determined. 12 Brachial measurements were normalized to the mean shear rate calculated from the first 10 seconds after cuff dilation. Similarly, femoral artery FMD was performed in the right common femoral artery 2 to 3 cm proximal to the bifurcation. Cuff placement was distal to the arterial injury site 5 mm above the popliteal fossa. The cuff was inflated for 5 minutes at 200 mm Hg, followed by rapid deflation. All brachial and femoral artery FMD procedures were performed in the Clinical Exercise Physiology Laboratory at the University of Florida by 2 experienced ultrasound technicians who had undergone previous training on this technique. The ultrasound images were measured by investigators blind to treatment allocation and stage of FMD test.

Graded Exercise Tests

All subjects performed symptom-limited maximum graded exercise tests on a treadmill with the use of a modified Naughton protocol before and after 35 sessions of EECP or sham. Primary measurements included time to angina, total exercise duration, and peak VO2. Criteria for termination of the symptom-limited maximum graded exercise tests included 2.5- to 3-mm ST-segment depression, respiratory exchange ratio >1, angina rated 2.5 to 3 on a 4-point scale, plateau of VO2, and volitional fatigue.
were performed for each variable to analyze the time point mean differences from baseline for each group and to determine within-group time point significance. Furthermore, Tukey post hoc analysis was performed with the use of the within-subject time point effect mean square error and test of between-subject effect mean square error derived from the primary repeated-measures ANOVA.

When significant differences between time points were observed for the EECP group, and there were no other significant differences between groups at baseline or between time points within the sham group, the significant $P$ values for the absolute mean changes are reported within groups by time point to simplify the presentation of the EECP treatment effect. Statistically significant absolute values are represented in the figure as percent changes from baseline, and Tukey post hoc significance values between groups and between time points are reported. Repeated-measures ANOVA between treatment and sham was used to analyze the descriptive patient characteristics, metabolic profile, cardiac intervention history, drug regimens, and graded exercise test results. These data are reported as mean±SD.

### Sample Size Calculation
A power analysis was performed to estimate the statistical power related to testing the following hypotheses in 30 patients: (1) EECP would improve endothelial function in peripheral muscular conduit arteries measured via FMD of the brachial artery; and (2) EECP would elicit commensurate changes in endothelial-derived vasoactive agent NOx. On the basis of the data of Shechter et al, for hypothesis 1, the posttreatment means were anticipated to be 8.2% and 3.1% for EECP and sham control, respectively. The anticipated SD was ~2.1% for the EECP group and 2.2% for the sham control group. A study of 20 evaluable EECP subjects and 10 evaluable sham controls will have 99% power on the basis of the Satterthwaite corrected $t$ test to have a $P$ value <5% (2-sided). On the basis of the data of Akhtar et al, for hypothesis 2, the posttreatment means are anticipated to be 43.9 and 27.1 μmol/L for EECP and sham control, respectively. The anticipated SD was ~7.5 μmol/L for the EECP group and 4.75 μmol/L for the sham control group. A study of 20 evaluable EECP subjects and 10 evaluable sham controls will have 99% power on the basis of the Satterthwaite corrected $t$ test to have a $P$ value <5% (2-sided).

### Results
All patients completed the entire EECP treatment or sham regimen, and there were no adverse cardiovascular events.

### Table 1. Patient Descriptive Characteristics and Metabolic Profile

<table>
<thead>
<tr>
<th></th>
<th>EECP (n=28)</th>
<th>Sham (n=14)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.44±9.63</td>
<td>64.57±9.63</td>
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<tr>
<td>Height, cm</td>
<td>172.77±21.61</td>
<td>172.77±21.61</td>
<td>0.360</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>92.60±17.24</td>
<td>92.42±17.32</td>
<td>0.518</td>
</tr>
<tr>
<td>Resting HR, bpm</td>
<td>59±7.8</td>
<td>61±9.2</td>
<td>0.454</td>
</tr>
<tr>
<td>Resting SBP, mm Hg</td>
<td>135±20.5</td>
<td>127±17.4†</td>
<td>0.004</td>
</tr>
<tr>
<td>Resting DBP, mm Hg</td>
<td>76±8.2</td>
<td>73±8.3*</td>
<td>0.024</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.88±11.15</td>
<td>30.80±11.02</td>
<td>0.460</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>121.46±46.76</td>
<td>144.62±92.29</td>
<td>0.973</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>148.42±70.89</td>
<td>176.08±101.57</td>
<td>0.263</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>142.69±37.37</td>
<td>142.08±23.86</td>
<td>0.829</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>69.83±33.43</td>
<td>67.83±28.89</td>
<td>0.962</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>39.31±16.36</td>
<td>37.08±16.20</td>
<td>0.379</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. HR indicates heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein. There were no significant differences ($P>0.05$) in patient descriptive characteristics or metabolic profile between EECP and sham groups at baseline. Significant values are reported from within-group repeated-measures ANOVA and Tukey post hoc analysis.

### Table 2. Baseline Patient Cardiac Intervention History and Drug Regimens

<table>
<thead>
<tr>
<th></th>
<th>EECP (n=28)</th>
<th>Sham (n=14)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior CABG</td>
<td>19 (76)</td>
<td>11 (79)</td>
<td>0.481</td>
</tr>
<tr>
<td>Prior PTCA</td>
<td>23 (92)</td>
<td>12 (88)</td>
<td>0.776</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>17 (61)</td>
<td>6 (43)</td>
<td>0.284</td>
</tr>
<tr>
<td>Multivessel CAD</td>
<td>25 (89)</td>
<td>13 (93)</td>
<td>0.718</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>21 (84)</td>
<td>11 (79)</td>
<td>0.804</td>
</tr>
<tr>
<td>Hypertension</td>
<td>23 (92)</td>
<td>12 (88)</td>
<td>0.776</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>26 (93)</td>
<td>14 (100)</td>
<td>0.317</td>
</tr>
<tr>
<td>Lipid-lowering drug</td>
<td>28 (100)</td>
<td>14 (100)</td>
<td>0.999</td>
</tr>
<tr>
<td>β-blocker</td>
<td>24 (86)</td>
<td>11 (79)</td>
<td>0.569</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>11 (39)</td>
<td>6 (43)</td>
<td>0.829</td>
</tr>
<tr>
<td>Long-lasting nitrates</td>
<td>25 (89)</td>
<td>12 (88)</td>
<td>0.744</td>
</tr>
<tr>
<td>ACE inhibition or ARB</td>
<td>26 (93)</td>
<td>12 (88)</td>
<td>0.469</td>
</tr>
<tr>
<td>Insulin</td>
<td>9 (32)</td>
<td>5 (36)</td>
<td>0.822</td>
</tr>
</tbody>
</table>

Data are presented as the number of patients per group and the percentage within each group (in parentheses). PTCA indicates percutaneous transluminal coronary angioplasty; ACE, angiotensin-converting enzyme; and ARB, angiotensin receptor blocker. There were no significant differences ($P>0.05$) in baseline characteristics, drug regimens, and cardiac intervention history between sham and EECP groups at baseline.
majority of patients (86%) were taking 3. Medications were not changed during the treatment period, and all patients waited until completion of the study before initiating exercise regimens that differed from activity levels at study entry. All vasodilator drugs were discontinued 12 hours before laboratory testing. Four patients in the EECP group (14%) and 2 patients in the sham group (14%) were insulin-dependent diabetics. None of the patients were current smokers.

**EECP Improved Brachial and Femoral Artery FMD**
At study entry, resting diameter and FMD (absolute diameter and Δ%) of the brachial and femoral arteries did not differ between groups. EECP therapy improved absolute brachial artery FMD (Figure 1A) and percent change in dilation (Figure 1C), and FMD normalized during the first 10 seconds after cuff release (0.18±0.05 to 0.30±0.11 and 0.20±0.07 to 0.21±0.07 normalized s\(^{-1}\) in EECP and sham, respectively; \(P<0.01\), treatment versus sham). EECP improved absolute femoral artery FMD (Figure 1B) and percent change in dilation (Figure 1D). No changes in brachial or femoral FMD occurred in the sham group.

**EECP Increased Plasma NOx and 6-Keto-PGF\(_1\alpha\) and Decreased ET-1**
At study entry, plasma levels of NOx, 6-keto-PGF\(_1\alpha\), and ET-1 did not differ between groups. EECP treatment increased NOx (22.8±9.8 to 31.9±11.1 and 23.9±16.1 to 24.3±14.5 μmol/L in EECP and sham, respectively; \(P<0.01\), treatment versus sham; Figure 2A), whereas ET-1 levels were concurrently decreased (2.2±0.8 to 1.5±1.3 and 2.1±0.9 to 2.1±0.9 pg/mL in EECP and sham, respectively; \(P<0.05\), treatment versus sham; Figure 2B). Consequently, the NOx:ET-1 ratio was improved (16.8±4.8 to 26.5±5.6 and 13.3±3.3 to 12.3±2.8 in EECP and sham, respectively; \(P<0.01\), treatment versus sham; Figure 2C) after EECP. The vasodilatory prostaglandin 6-keto-PGF\(_1\alpha\) increased (108±71 to 185±111 and 118±60 to 120±94 pg/mL in EECP and sham, respectively; \(P<0.05\), treatment versus sham; Figure 2D) after EECP. Plasma levels of NOx, 6-keto-PGF\(_1\alpha\), ET-1, and the NOx:ET-1 ratio did not change in the sham group.

**EECP Decreased Inflammatory Cytokines and Endothelial Adhesion Molecules**
At study entry, plasma levels of TNF-α, hsCRP, MCP-1, and sVCAM did not differ between groups. EECP therapy decreased TNF-α (6.1±2.2 to 5.0±1.7 and 6.2±1.2 to 6.4±1.5 pg/mL in EECP and sham, respectively; \(P<0.01\), treatment versus sham; Figure 3A), hsCRP (3.4±2.17 to 2.3±1.8 and 3.4±0.9 to 3.5±1.1 mg/L in EECP and sham, respectively; \(P<0.05\), treatment versus sham; Figure 3B), MCP-1 (227±74 to 171±43 and 230±92 to 231±75 pg/mL in EECP and sham, respectively; \(P<0.001\), treatment versus sham; Figure 3C), and sVCAM (917±406 to 821±317 and 955±256 to 967±230 ng/mL in EECP and sham, respec-
tively; \( P<0.05 \), treatment versus sham; Figure 3D). No changes in inflammatory cytokines or endothelial adhesion molecules were observed in the sham group.

**EECP Improved Redox Balance**

At study entry, plasma levels of 8-iso-PGF\(_2\alpha\), ADMA, and SOD did not differ between groups. EECP decreased 8-iso-PGF\(_2\alpha\) (1709±594 to 1356±411 and 1720±674 to 1696±472 pg/mL in EECP and sham, respectively; \( P<0.01 \), treatment versus sham; Figure 4A) and ADMA (0.64±0.18 to 0.46±0.18 and 0.66±0.11 to 0.66±0.22 \( \mu \)mol/L in EECP and sham, respectively; \( P<0.001 \), treatment versus sham; Figure 4B). SOD did not change significantly after EECP (1.59±0.39 to 1.49±0.48 and 1.54±0.68 to 1.46±0.66 U/mL in EECP and sham, respectively; \( P>0.05 \), treatment versus sham; Figure 4C). Levels of 8-iso-PGF\(_2\alpha\), ADMA, and SOD did not change in the sham group.

**EECP Decreased Peripheral Blood Pressure**

Peripheral blood pressure and heart rate information is presented in Table 1. We observed no significant changes in resting heart rate in either group. Resting systolic and diastolic brachial pressures were decreased after 35 sessions of EECP. No changes in blood pressure were observed in the sham group.

**EECP Increased Peak \( \dot{V}O_2 \), Exercise Duration, and Time to Angina**

At study entry, time to angina, peak \( \dot{V}O_2 \), total exercise duration, peak respiratory exchange ratio, heart rate, systolic blood pressure, and rating of perceived exertion did not differ between groups during the symptom-limited maximum graded exercise tests (Table 3). After 35 sessions of EECP, time to angina, peak \( \dot{V}O_2 \), and total exercise duration were increased. No changes in symptom-limited maximum graded exercise test parameters were observed in the sham group.
EECP Improved Functional Classification

EECP led to an improvement by 1 Canadian Cardiovascular Society (CCS) class in 2 patients (7%), by 2 classes in 19 patients (68%), and by 3 classes in 6 patients (21%). One patient did not change CCS class. Average CCS classification (3.16±0.47 to 1.20±0.40 and 2.93±0.26 to 2.93±0.26 in EECP and sham, respectively; P<0.001, treatment versus sham), number of anginal episodes per day (1.8±1.47 to 0.5±0.70 and 1.7±1.39 to 1.6±1.24 in EECP and sham, respectively; P<0.01, treatment versus sham), and daily nitrate usage (1.1±1.44 to 0.2±0.41 and 1.0±1.12 to 0.9±1.11 in EECP and sham, respectively; P<0.01, treatment versus sham) decreased in the EECP group. There were no changes in any measure of angina symptom reduction in the sham group.

EECP and Peripheral Flow-Mediated Dilation

This prospective, randomized sham-controlled study demonstrates for the first time, in the same cohort of CAD patients, that EECP intervention (1) improves brachial and femoral artery FMD; (2) increases bioavailability of nitric oxide and PGF1; (3) decreases plasma biomarkers of inflammation, oxidative stress, and ET-1; (4) decreases angina symptoms; and (5) increases exercise tolerance. These EECP-induced clinical benefits occurred in CAD patients with refractory angina pectoris who were receiving optimal medical therapy.

Flow-Mediated Vasodilation

The primary focus of the present study was the direct effects of episodic changes in shear stress, as delivered during repeated bouts of EECP, on peripheral muscular conduit artery FMD. FMD in the brachial and femoral arteries was increased by 51% and 30%, respectively (Figure 1). These arterial adaptations appear to be the collective result of our observed dramatic increases in NOx and PGF1 and concurrent decreases in the vasoconstrictor ET-1.

Endothelial-Derived Vasoactive Agents

Endothelial dysfunction in patients with CAD is manifest by decreased secretion of paracrine vasodilators, including nitric oxide and PGF1, or an increased production of the potent vasoconstrictor ET-1, and/or resistance of vascular smooth muscle to nitric oxide. In the present study, 35 sessions of EECP increased plasma levels of NOx by 36%, whereas ET-1 levels were concurrently decreased by 25% (Figure 2). ET-1 values after EECP treatment compare favorably with reference values (1 to 1.5 pg/mL) in normal healthy humans.15,16 Previously, Zhang and coworkers17 reported that EECP increased the expression of endothelial nitric oxide synthase in hypercholesterolemic pigs, and small uncontrolled studies have also reported increased plasma levels of the potent endothelial-derived vasodilator PGF1, the stable metabolite of prostacyclin. We observed a 28% reduction in plasma ADMA levels after EECP (Figure 4). This finding is similar to findings after exercise training in type 1 diabetics18 and CAD patients.19 ADMA can be

**Discussion**

This was the first study to examine the mitigating effects of EECP on circulating levels of the potent endothelial-derived vasodilator PGF1, the stable metabolite of prostacyclin. We found a 171% increase in PGF1 after EECP (Figure 2). This was also the first study to measure the effects of EECP on plasma levels of ADMA, the major endogenous competitive inhibitor of nitric oxide synthase. We observed a 28% reduction in plasma ADMA levels after EECP (Figure 4). This finding is similar to findings after exercise training in type 1 diabetics18 and CAD patients.19 ADMA can be
increased up to 10-fold greater than normal in patients with CAD.20 ADMA contributes to endothelial dysfunction by displacing the substrate l-arginine from the catalytic site of nitric oxide synthase, thus inhibiting nitric oxide formation and reducing nitric oxide bioavailability. Oxidative stress is a key modulator of ADMA levels,20 suggesting that our observed 21% reduction in the marker of oxidative stress (8-iso-PGF2α; Figure 4) may have led to decreased levels of ADMA.

Plasma Markers of Inflammation
Endothelial function is adversely affected by inflammation, and extensive evidence indicates that patients with CAD have elevated levels of proinflammatory cytokines and adhesion molecules,29 including TNF-α, hsCRP, MCP-1, and sVCAM. Moreover, CAD patients with refractory angina symptoms have proinflammatory biomarker levels that are elevated even further.30 Increased plasma levels of TNF-α, hsCRP, and MCP-1 are known to predict future coronary events. In vitro, CRP and TNF-α also actively participate in the atherogenic process by contributing to decreased nitric oxide synthase production and increased endothelial cell activation characterized by expression of sVCAM. After EECP, we observed reductions in plasma levels of TNF-α (−16%), hsCRP (−32%), MCP-1 (−13%), and sVCAM (−6%) (Figure 3). The anti-inflammatory effects of EECP may be clinically relevant with regard to decreasing the risk for future cardiovascular events in this patient population. For instance, sVCAM levels >1130 ng/mL are associated with increased ischemic events and mortality in patients with CAD.31,32 In the present study, 6 of 28 patients in the intervention group had baseline sVCAM >1130 ng/mL. After EECP, only 1 of 28 patients had sVCAM values >1130 ng/mL. Similarly, EECP shifted mean CRP values from the high-risk (>3 mg/L) to the moderate-risk category (1 to 3 mg/L).33 Despite significant reductions, TNF-α levels after EECP remained high and approximated the 95th percentile of healthy control values.34 Increased bioavailability of nitric oxide after EECP therapy is the likely mechanism responsible for reduced plasma inflammatory markers. Nitric oxide serves an anti-inflammatory role by inhibiting the expression of MCP-1 and reducing VCAM-1 expression.35

Lipid Peroxidation and Antioxidant Capacity
This was the first study to examine the effects of EECP on lipid peroxidation. We observed a 21% decrease in plasma levels of 8-iso-PGF2α (Figure 4). F2-isoprostanes are a class

### Table 3. Results From the Symptom-Limited Graded Exercise Tests

<table>
<thead>
<tr>
<th></th>
<th>EEC (n=28)</th>
<th>Sham (n=14)</th>
<th>P, Between-Group Comparison</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>Peak V̇O₂, mL/kg per minute</td>
<td>17.0±6.0</td>
<td>19.4±6.9*</td>
<td>16.5±4.7</td>
</tr>
<tr>
<td>Peak exercise duration, s</td>
<td>586.0±193.5</td>
<td>773.6±263.2*</td>
<td>597.1±181.6</td>
</tr>
<tr>
<td>Peak time to angina, s</td>
<td>406.1±184.6</td>
<td>645.1±297.8†</td>
<td>449.4±202.9</td>
</tr>
<tr>
<td>Peak angina rating</td>
<td>2.5±0.9</td>
<td>1.7±1.1*</td>
<td>2.3±1.2</td>
</tr>
<tr>
<td>Peak heart rate, bpm</td>
<td>112.4±13.8</td>
<td>117.4±18.9</td>
<td>115.6±20.1</td>
</tr>
<tr>
<td>Rating of perceived exertion</td>
<td>16.1±1.8</td>
<td>16.8±1.9</td>
<td>16.6±1.7</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.99±0.08</td>
<td>1.04±0.08</td>
<td>0.98±0.12</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. T1 indicates time 1 (baseline); T2, time 2 (after 35 sessions of EECP). There were no significant differences between EECP and sham groups at baseline. Significance values are also reported from within-group repeated-measures ANOVA and Tukey post hoc analysis.

*P<0.01, †P<0.001.
of prostaglandin-like compounds that are produced by free radical–mediated lipid peroxidation of arachidonic acid, independent of cyclooxygenase. One of these isoprostanes, 8-iso-PGF$_{2\alpha}$ is viewed as the most valid plasma marker to assess systemic oxidative stress. 8-Iso-PGF$_{2\alpha}$ is a potent vasoconstrictor and an independent risk factor associated with CAD. The mechanisms responsible for decreased 8-iso-PGF$_{2\alpha}$ are unclear because EECp did not change activity levels of the extracellular antioxidant enzyme SOD (Figure 4). However, nitric oxide has been shown to have antioxidative properties, and our observed 36% increase in plasma NOx after EECp could suppress oxidative stress.

**Angina Symptoms**

Our evidence of improved arterial FMD and endothelial-derived vasoactive agents after EECp was paralleled by decreased angina symptoms. Patients reduced their CCS angina classification from 3.1 to 1.2 (mean±SEM), daily anginal episodes by \( \pm 72\% \), and daily nitrate usage by 81%. The antiangiinal benefits of EECp are perhaps best illustrated by the fact that 7 patients in the intervention group completed their exit symptom-limited maximum graded exercise tests without any symptoms of angina. In contrast, each of those 7 patients experienced angina levels between 2 and 3 (on the 4-point scale) on their symptom-limited maximum graded exercise tests at study entry.

**Clinical Relevance**

We believe that the present findings have important clinical relevance. In the United States alone, >2.5 million patients with symptomatic CAD are not amenable to percutaneous coronary intervention or CABG because of unsuitable coronary anatomy, multiple previous revascularization attempts, age, additional comorbid conditions, or patient preference. For those patients in whom repeat (or initial) revascularization procedures are not appropriate and in whom aggressive medical therapy fails to reduce anginal pain, the search for alternate therapeutic options continues. EECp is the only truly noninvasive, atrumatic intervention for which a reduction of angina symptoms and nitrate usage, increased exercise tolerance, and an improvement in myocardial ischemia have been shown.

**Study Limitations**

Reduced sympathetic tone during the 35 sessions of EECp could independently improve peripheral artery reactivity. However, our plasma norepinephrine results indicate that sympathetic tone was unchanged after 35 sessions of EECp. Changes in metabolic profile could also confound the findings of the present study. However, we found no differences in lipid profile between groups at study entry, and there were no changes in total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, and glucose levels during the 7 to 8 weeks of the study (Table 1). Finally, we did not perform endothelium-independent reactivity tests. However, it is unlikely that our observed improvements in FMD and limb blood flow are due to heightened smooth muscle sensitivity to nitric oxide or altered cyclic guanosine monophosphate signaling during EECp treatment. Indeed, it was reported previously that effects of sublingual nitroglycerine spray, a nitric oxide donor acting directly on smooth muscle cells, are unchanged in CAD patients after EECp therapy.

**Summary**

In summary, by demonstrating that thirty-five 1-hour sessions of EECp improve brachial and femoral artery FMD, results from the present sham-controlled study support the hypothesis that EECp improves peripheral arterial function in patients with symptomatic CAD. Our hypothesis is further supported by novel neurohormonal evidence that EECp markedly increases plasma NOx and 6-keto-PGF$_{1\alpha}$ and simultaneously decreases ET-1, 8-iso-PGF$_{2\alpha}$, and the proinflammatory markers TNF-\( \alpha \), hsCRP, MCP-1, and sVCAM.

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**Disclosures**

None.

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