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ENHANCED EXTERNAL COUNTERPULSATION IMPROVES ENDOTHELIAL FUNCTION AND EXERCISE CAPACITY IN PATIENTS WITH ISCHEMIC LEFT VENTRICULAR DYSFUNCTION

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Abstract

Enhanced external counterpulsation (EECP) therapy decreases angina episodes and improves quality of life in patients with left ventricular dysfunction (LVD). However, studies have not elucidated the mechanisms of action and overall effects of EECP in patients with LVD. The purpose of this study was to investigate the effects of EECP on endothelial function in peripheral conduit arteries and exercise capacity (peak VO₂) in patients with LVD. Patients with ischemic LVD (EF 34.5±4.2%; n=9), and patients with symptomatic CAD and preserved LV function (EF 53.5±6.6%; n=15), were studied before and after 35 1-hr sessions of EECP. Brachial (bFMD) and femoral (fFMD) artery flow-mediated dilation were evaluated using high-resolution ultrasound. EECP elicited similar significant improvements in the following FMD parameters amongst the CAD and LVD groups, respectively: absolute bFMD (+53% and +70%); relative bFMD (+50% and +74 %); bFMD normalized for shear rate (+70% and +61%); absolute fFMD (+33% and +21%); and relative fFMD (+32% and +17%) (*P* 0.05 between groups). EECP significantly improved plasma levels of nitrate/nitrite (NOx) (+55% and +28%; μmol/L) and prostacyclin (6-keto-PGF_{1α}) (+50% and +70%); and improved peak VO₂ (+36% and +21%), similarly in both the CAD and LVD groups, respectively; (*P* 0.05 between groups). Despite reduced LV function, EECP therapy significantly improved peripheral vascular function and functional capacity similar in magnitude to that observed in CAD patients with preserved LV function.

Keywords

coronary artery disease; endothelial function; enhanced external counterpulsation; left ventricular dysfunction; vasoactive biomarkers

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DISCLOSURE

The authors declare no conflicts of interest.

INTRODUCTION

The functional integration of the heart chambers, vascular wall, endothelium, and systemic neurohormones regulate oxygen and blood delivery to the tissues of the body. Delivery of blood and oxygen are affected by left ventricular dysfunction (LVD) and heart failure (HF) both directly and indirectly through activation of neurohormonal systems (renin-angiotensin-aldosterone-system, sympathetic nervous system, and atrial natriuretic peptides), which exert their effects on the heart, vascular wall, and endothelium. Presently, there is no single pharmacologic treatment capable of concurrently increasing cardiac contractility and lowering vascular resistance in patients with compromised ejection fraction (EF).⁽¹⁾ Moreover, the clinical benefits from primary pharmacotherapy are maintained only while medication is being taken and there are no lasting long-term benefits after drug treatment is discontinued. In optimally medicated patients with LVD, one study found that enhanced external counterpulsation (EECP) therapy has increased cardiac output during treatment by more than 75% and reduced systemic vascular resistance by 20%–30%.⁽²⁾ Further, these improvements in cardiac output and systemic vascular resistance were superior to any reported responses to oral or intravenous vasodilators and some research suggests that the beneficial effects of EECP persist long after therapy is completed.^(1, 2)

EECP is a U.S. Food and Drug Administration approved, non-invasive outpatient therapy for the treatment of patients with coronary artery disease (CAD) and refractory angina pectoris who fail to respond to standard medical treatment. Acutely, EECP has been shown to improve diastolic filling, decrease left ventricular (LV) end-diastolic pressure, improve LV time to peak filling rate, and increase LV end-diastolic volume.⁽³⁾ In addition, EECP has been shown to reduce wasted LV energy, myocardial oxygen demand, central and peripheral arterial stiffness and improve conduit artery endothelial function in patients with coronary artery disease (CAD) and preserved LV function.^(4, 5) Importantly, EECP has been shown to have beneficial effects on central hemodynamics in patients with symptomatic or refractory LVD and may represent the most effective non-invasive adjuvant therapy for the treatment of angina pectoris in patients with LVD.⁽⁶⁾ Indeed, the International EECP Patient Registry has shown that EECP treatment decreased angina episodes and improved quality of life in patients with severe LVD (ejection fraction < 35%).⁽⁷⁾ To date, however, studies have not elucidated the mechanism of action and the effects of EECP therapy in patients with LVD. Therefore, the purpose of this study was to investigate the effects of EECP on endothelial function in peripheral muscular conduit arteries, and the concurrent alterations in exercise tolerance and angina classification in CAD patients with and without LVD.

RESULTS

All subjects completed the entire EECP treatment protocol without adverse events. Subject descriptive characteristics and metabolic profile are presented in Table 1. By design, EF was lower in the LVD group (EF 34.5±4.2%) than in the CAD group (EF 53.5±6.6%), ($P<0.001$). Table 2 contains cardiac intervention history and drug regimens. There were no differences between the CAD and LVD groups at study entry with respect to blood pressure, drug therapy, previous cardiovascular history, cardiovascular risk factors, revascularization

procedures and metabolic profile ($P>0.05$). The majority of patients were male (79%), had a long history of CAD, CAD related interventions and were not candidates for further revascularization therapy. Pharmacotherapy was not altered during the treatment period, and all patients waited until completion of the study before initiating exercise regimens that differed from pre-treatment activity levels. None of the patients were current smokers.

EECP Improved Resting Blood Pressure and Functional Classification

Peripheral blood pressures were not different between CAD and LVD groups before EECP therapy ($P>0.05$). Systolic blood pressure was significantly and comparably reduced after EECP therapy in both the CAD and LVD groups (Table 1). Canadian Cardiovascular Society angina classification was reduced comparably in the CAD and LVD groups after EECP treatment (Table 1).

EECP Improved Brachial and Femoral Artery FMD

At study entry, resting diameter and FMD (absolute diameter and %) of the brachial and femoral arteries did not differ between groups ($P>0.05$) (Figures 1 and 2). EECP treatment significantly ($P<0.05$) improved the following FMD parameters comparably in both the CAD and LVD groups, respectively: absolute bFMD (0.213 ± 0.05 mm to 0.325 ± 0.04 mm and 0.226 ± 0.05 mm to 0.385 ± 0.04 mm); relative bFMD (4.12 ± 1.14 % to 6.16 ± 1.06 % and 4.18 ± 1.24 % to 7.26 ± 1.17 %); bFMD normalized for shear rate (0.185 ± 0.05 s⁻¹ to 0.313 ± 0.06 s⁻¹ and 0.191 ± 0.06 s⁻¹ to 0.307 ± 0.07 s⁻¹); absolute fFMD (0.198 ± 0.06 mm to 0.264 ± 0.07 mm and 0.194 ± 0.06 mm to 0.236 ± 0.08 mm); relative fFMD (2.77 ± 0.16 % to 3.66 ± 0.19 % and 2.73 ± 0.17 % to 3.19 ± 0.20 %) (Figures 1 and 2). No changes in resting brachial or femoral artery diameters were observed in either group (Figures 1 and 2). In addition, relative bFMD (%) was significantly associated with exercise duration in both the CAD and LVD groups combined after EECP therapy (Figure 4).

EECP Increased Vasoactive Substances

At study entry, plasma levels of NOx and 6-keto-PGF_{1α} did not differ between groups ($P>0.05$) (Figure 3). EECP treatment significantly improved plasma levels of NOx (19.4 ± 1.54 to 30.1 ± 1.62 ; μmol/L and 22.0 ± 1.67 to 28.2 ± 1.72 ; μmol/L) and 6-keto-PGF_{1α} (119 ± 18.0 pg/ml to 179 ± 25 pg/ml and 111 ± 19 pg/ml to 188 ± 28.3 pg/ml) similarly in both the CAD and LVD groups, respectively (Figure 3). Further, NOx was significantly associated with relative bFMD (%) in both the CAD and LVD groups combined following EECP treatment (Figure 4).

EECP Improved Exercise Tolerance and Peak VO₂

At study entry, peak VO₂ and exercise duration were lower in the LVD group compared with those in the CAD group ($P<0.05$) (Table 3). EECP significantly improved the following GXT parameters comparably in the CAD and LVD groups: peak VO₂, exercise duration, time to angina, and angina rating at peak exercise in both the CAD and LVD groups (Table 3). After EECP treatment peak VO₂ and exercise duration remained lower in the LVD group when compared to that in the CAD group ($P<0.05$) (Table 3).

DISCUSSION

The International EECPP Patient Registry reported that EECPP treatment decreases angina episodes and improves quality of life in patients with severe LVD (ejection fraction < 35%) and that these improvements can last up to 5 years.⁽⁷⁾ To date, however, studies have not elucidated the mechanism of action and the overall effects of EECPP therapy in patients with LVD. The present stratified, single-blinded prospective study demonstrates that EECPP improves brachial and femoral artery endothelial function, maximal aerobic capacity, and NO and prostaglandin bioavailability similarly in CAD patients with and without LVD. Moreover, the current study suggests that increases in relative brachial FMD (%) in response to EECPP therapy is positively associated with NOx bioavailability and exercise duration.

Conduit Artery Endothelial Function

Flow mediated dilation is an independent predictor of coronary heart disease.⁽⁸⁾ Improved endothelial function is associated with reduced morbidity and mortality in heart failure patients.⁽⁹⁾ In a study to determine the interrelationships of surrogate measures of endothelial function (bFMD), and measures of arterial stiffness and arterial compliance, Koyoshi and colleagues determined that bFMD is the best predictor of the severity of CAD independent of traditional coronary risk factors in CAD patients with stable angina pectoris.⁽¹⁰⁾ The present study demonstrates that EECPP improves peripheral conduit artery endothelial function similarly in CAD patients with and without LVD. EECPP therapy improved absolute brachial artery flow mediated dilation (bFMD) by 53% and 70% in the CAD and LVD groups, respectively. Moreover, bFMD normalized to shear rate was similarly increased in both the CAD and LVD groups resulting in nearly identical improvements (+69% and +61%, respectively). Similarly, femoral artery flow mediated dilation (fFMD) was increased in the CAD and LVD group by 33% and 21%, respectively, further demonstrating the systemic improvements in conduit artery reactivity.

Endothelial-Derived Vasoactive Agents

Endothelial dysfunction in patients with CAD is characterized by either decreased bioavailability of paracrine vasodilators including nitric oxide and prostacyclin and/or resistance of vascular smooth muscle to NO. The present study demonstrates that 35 1 hour sessions of EECPP increases plasma NOx (+55% and 28%) and 6-keto-PGF_{1α} (+51% and +70%) similarly in CAD patients with and without LVD, respectively. These findings are similar to those previously reported.^(4, 11–13)

Mechanisms of Improved Endothelial Derived-Vasoactive Agents and Function

Each session of EECPP may be thought of as providing a direct dose of vascular medicine via the significant increases in pulsatile and oscillatory flow during EECPP treatment and may provide a form of ‘massage’ on the endothelium improving its function through local endothelial cell and systemic mechanisms.⁽¹⁴⁾ Specifically, EECPP treatment produces two opposite blood flow patterns, antegrade flow in the brachial artery and retrograde flow in the femoral artery, which increase shear stress in the brachial and femoral arteries by 75% and 402%, respectively.⁽¹⁵⁾ Further, bFMD and fFMD are acutely increased after a single bout of EECPP suggesting that dramatic increases in shear stress created by EECPP improves

endothelium dependent arterial vasodilation in muscular conduit arteries.⁽¹⁵⁾ Repeated bouts of increased shear stress may be responsible, in part, for the observed improvement in conduit artery endothelial function and increased nitric oxide bioavailability in CAD patients with and without LVD following EECF therapy. Indeed, significant moderate relationships were observed between relative bFMD (%), NO, and functional capacity after EECF treatment suggesting that improvement in endothelial function following EECF may be associated with improved functional capacity in patients with moderate LV systolic dysfunction.

Recently, Kozdag and colleagues, investigated the effects of EECF therapy on prognostic markers of heart failure in CAD patients with ischemic heart failure (LVEF; $30 \pm 13\%$).⁽¹⁶⁾ They report significant improvements in New York Heart Association (NYHA) functional classification, left ventricular ejection fraction, B-natriuretic peptide and uric acid levels, free-T3/free-T4 ratio, and mitral annular E velocity.⁽¹⁶⁾ During EECF therapy, cuff inflation leads to aortic diastolic pressure augmentation, resulting in increased diastolic coronary pressure and increased coronary blood flow; whereas cuff deflation results in left ventricular systolic unloading.⁽¹⁷⁾ Ultimately, this pattern of pneumatic cuff inflation and deflation acutely elicits a decrease in systemic vascular resistance and afterload resulting in an increase in cardiac output.^(18–20) In addition, EECF acutely raises right atrial pressure, leading to increased preload to the left ventricle.⁽²¹⁾ Moreover, myocardial contractility and systolic function are acutely improved during EECF treatment in CAD patients with refractory angina.⁽²²⁾ Chronically, EECF therapy improves diastolic filling, decreases left ventricular end-diastolic pressure, and improves LV peak filling rate, end-diastolic volume, and time to peak filling rate.⁽³⁾ EECF therapy reduced Canadian Cardiovascular Society angina classification (CCS) similarly in both the CAD and LVD groups concurrently with improvement in endothelial function. Based on their findings, Kozdag and colleagues concluded that EECF treatment improves clinical and biochemical parameters which are prognostic markers in symptomatic CAD patients with LVD.⁽¹⁶⁾ In the current study, we report similar reductions in functional classification after EECF in CAD patients with or without LVD.

Graded Exercise Testing

EECF therapy is reported to improve LVEF by 3–4% in subjects similar to those in the current study.⁽¹⁶⁾ Further, a ~4% improvement in LVEF is associated with a 47% increase in maximal aerobic capacity in patients with HF.⁽²³⁾ Indeed, in the present study EECF therapy increased maximal aerobic capacity, exercise duration, and time to anginal symptoms during exercise while reducing overall self perceived anginal rating at maximal exertion (Table 3). EECF improved exercise duration similarly in both the CAD and LVD groups by an average greater than 3 minutes with only 2 patients, 1 in the CAD and 1 in the LVD group, increasing their total exercise time by less than 1 minute (Table 3).

The findings of the current study are somewhat similar to those reported from the Prospective Evaluation of EECF in Congestive Heart Failure (PEECH) trial where improvements in exercise tolerance, quality of life, and NYHA functional classification were observed after EECF therapy in patients with mild-moderate CHF (EF =

26.3±6.3%).⁽²⁴⁾ However, the PEECH Trial reported that the improvements in exercise duration and functional classification were unaccompanied by significant increases in peak VO₂ following EECp therapy in patients with CHF.⁽²⁴⁾ In contrast, we observed comparable increases in peak VO₂ by 36% and 21%, exercise duration by 32% and 53%, and time to angina by 63% and 57% in both the CAD and LVD groups, respectively.

The functional improvements in response to optimal pharmacotherapy, exercise interventions, and combinations of both for the treatment of LVD and CHF have been better established.^(25–30) Poelzl and colleagues suggest that optimized neurohormonal therapy (combined angiotensin converting enzyme (ACE) inhibitors and beta-blockers) improves submaximal exercise capacity by 45% and bFMD by 34% in patients with CHF.⁽²⁵⁾ Further, the improvement in functional capacity in response to optimal pharmacotherapy with ACE inhibition and beta-blockade is significantly associated with the increase in conduit artery endothelial function.⁽²⁵⁾ In a study investigating the effects of sildenafil, Lewis et al., observed a 14% increase in peak VO₂.⁽²⁶⁾ Digoxin has been associated with a 14% increase in exercise duration.⁽²⁸⁾ In the current study, all patients were receiving, and continued to receive, optimal pharmacologic therapy, which included ACE inhibition and beta-blockade (Table 2), as determined by their cardiovascular physician during their participation in this study. Further, no changes in drug regimen were reported in any patient during their study participation. Therefore, the findings of the current study suggest that the beneficial effects of EECp therapy on functional capacity, conduit artery endothelial function, and vasoactive substances may be additive to the proven efficacy of optimal drug therapy.

In comparison, the functional improvements after EECp observed in the LVD group in the present study are similar to those reported by Hambrecht et al. after 6 month's of endurance exercise training therapy in patients with chronic heart failure.^(29, 30) Regular physical exercise results in basal endothelial NO formation and endothelial dependent vasodilation in peripheral conduit arteries in patients with CHF.⁽²⁹⁾ Hambrecht and colleagues also report 26% and 32% increases in peak VO₂ and exercise duration, respectively, which parallels improvement in functional classification following a 6 month exercise intervention.⁽³⁰⁾ The possible added benefits of enrollment in an endurance exercise training program immediately after EECp treatment completion in CAD patients with chronic stable angina and moderate LV systolic dysfunction is unknown.

Commensurate with the improvements in exercise capacity, EECp reduced patient reported rating of perceived angina at maximal exertion by 39% and 35% in both CAD and LVD groups, respectively. Soran and colleagues reported a 7.45% increase in peak oxygen uptake (14.99ml/kg/min to 15.98 ml/kg/min) 1 week after EECp treatment in patients with LVD (LVEF = 23.3±7.8%) and at 6 month follow-up oxygen uptake was further increased to 27.1% (14.78 to 18.41 ml/kg/min) above baseline.⁽²⁾ These data suggest that the beneficial effects of EECp on exercise capacity and quality of life persist after therapy completion and may be due to continued vascular alterations and/or increases in activity that promote cardiovascular function.⁽²⁾ In contrast, the clinical and functional benefits from primary pharmacotherapy are maintained only while medication is being taken and there are no lasting long-term benefits after drug treatment is discontinued. Notwithstanding, patients typically report improvement in angina symptoms, increased activity levels, and decreased

nitroglycerin usage after EECp therapy. Thus, EECp may be thought of as a 'bridge' to exercise in CAD patients with LVD or mild heart failure who could not previously tolerate periods of sustained exertion without experiencing anginal symptoms.

Experimental Considerations and Future Directions

Endothelium-independent reactivity tests were not performed. However, it is unlikely that our observed improvements in FMD are due to heightened smooth muscle sensitivity to nitric oxide or altered cyclic guanosine monophosphate signaling during EECp treatment. Indeed, it was previously reported that effects of sublingual nitroglycerine spray, a nitric oxide donor acting directly on smooth muscle cells, are unchanged in CAD patients after EECp therapy.⁽³¹⁾ Left ventricular ejection fraction was not measured after EECp treatment. However, in the present study LVEF in the LVD groups were similar at baseline to those investigated by Kozdag and colleagues and we can only conclude that the patients in the current study responded similar to those in the previous study.⁽¹⁶⁾ Major limitations of the present study are the small sample size and the absence of a control or Sham EECp group. Therefore, the possibility of a training effect or over estimation of the treatment effect cannot be entirely excluded. However, all participants were instructed to maintain pre-study activity levels and all patients waited until completion of the study before initiating exercise regimens that differed from pre-treatment activity levels. Importantly, patients who participated in the current study were referred for EECp treatment by a physician and required EECp therapy to reduce chronic anginal symptoms. Our laboratory, and others, have previously shown that time-control groups or groups receiving 35 sessions of Sham EECp therapy at lower than treatment inflation pressures (70 mmHg) and presented no measureable hemodynamic changes or differences in pre- and post-treatment dependent variable values.^(4, 32) Therefore, it was determined during the design of the present study to exclude a control or Sham EECp group and not defer necessary therapy for this cohort of patients.

Summary

EECP treatment improves brachial and femoral artery endothelial function and NO_x levels similarly in patients with CAD possessing preserved LV function and in patients with LV dysfunction. Further, EECp treatment improves peak VO₂, total exercise time, exercise time to angina, and CCS angina classification to the same magnitude in patients with CAD possessing preserved LV function and in patients with LV dysfunction.

Despite moderate LV systolic dysfunction, significant improvements in peripheral vascular function and functional capacity are realized following a standard course of EECp therapy. This indicates that EECp may be a viable treatment option to improve not only quality of life, but also functional capacity (peak VO₂) and peripheral vascular function in HF patients who are not amenable to surgical and/or pharmacological intervention. Further, the beneficial effects of EECp therapy on peripheral vascular function suggests that EECp may be effective as adjuvant therapy for the treatment of angina pectoris in patients with LVD combined with the proven efficacy of current pharmacotherapy. Our data indicate that EECp serves as 'bridge to exercise' and may be a useful intervention in individual patients with LV dysfunction who are poor candidates for aerobic exercise training and that peripheral

vascular adaptations may be the specific mechanisms responsible for the beneficial clinical effects of EECP in patients with LV dysfunction.

METHODS

Baseline Status of Subjects

Twenty-four (n=24) consecutive patients with chronic stable angina referred for EECP treatment were enrolled in this study and stratified by left ventricular ejection fraction (LVEF) into either a CAD with preserved LV function group (EF $\geq$ 40%, n = 15 (CAD)) or a group with ischemic etiology of moderate LV systolic dysfunction (EF $>$ 30%, but $<$ 40%, n = 9 (LVD)). Male and female patients were referred for EECP therapy due to the presence of chronic angina for greater than 3 months secondary to myocardial ischemia in the presence of angiographic multi-vessel CAD that could not be controlled by a combination of medical therapy, angioplasty/stent, and/or coronary artery bypass graft (CABG) surgery. All patients were receiving, and continued to receive, optimal pharmacologic therapy as determined by their cardiovascular physician during participation in this study. Further, no changes in drug regimen were observed in any patient during their study participation throughout. This study was approved by the Institutional Review Board of the University of Florida and written informed consent was obtained from all subjects.

Exclusion Criteria

Patients were excluded from the study if they met any of the following criteria: absence of ST segment depression (1 mm minimum) during graded exercise testing; $>$ 75 years of age; CABG within past 3 months or percutaneous coronary intervention in past 6 months; cardiac catheterization for any reason within past 2 weeks; cardiac arrhythmia that would significantly interfere with triggering of the EECP device; symptomatic heart failure and/or LV ejection fraction $<$ 30%; decompensated heart failure; severe aortic regurgitation; valvular heart disease; ICD if triggered within past 6 months, history of deep vein thrombosis; uncontrolled hypertension; pregnancy; pulmonary congestion; or systemic hypotension.

EECP Treatment

An EECP (Vasomedical, Westbury, New York) therapeutic system consists of an air compressor, a treatment table, a control console and an integrated set of pneumatic cuffs, and has been described previously.⁽¹⁴⁾ Briefly, cuffs that are positioned on the calves, thighs and upper thighs/buttocks are inflated with compressed air to target inflation pressures of 300 mm Hg sequentially from distal to proximal in early diastole and rapidly deflated immediately prior to the onset of systole. Inflation and deflation of the cuffs is triggered by events in the cardiac cycle via microprocessor-interpreted electrocardiogram signals. LVD and CAD groups were studied before and after a standard course of EECP treatment consisting of 35 1-hr sessions 5 days a week for 7 weeks.

Blood Collection and Biochemical Assays

Venipuncture was performed before and after 35 sessions of EECP. Serum lipids and glucose were measured in hospital laboratories by validated techniques. Plasma levels of

endothelial derived 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PG $F_{1\alpha}$), the major stable metabolite of the vasodilator prostacyclin was measured. Measurement of the stable nitric oxide metabolites, nitrate and nitrite (NOx), was used to estimate nitric oxide (NO) bioavailability. To minimize the influence of dietary nitrates, all subjects kept a diet diary and followed the National Institutes of Health low nitrate diet guidelines for a minimum of 48 hours prior to each blood draw.⁽³³⁾ NOx and 6-keto-PGF $_{1\alpha}$, were measured by commercial assays (Cayman, Ann Arbor, MI, USA). The intra- and inter assay coefficients of variance were 3.2% and 5.0% for NOx, and 5.5% and 8.1% for 6-keto-PGF $_{1\alpha}$, respectively. Biochemical assays were performed in 1 batch after all subjects had completed the study.

Peripheral FMD

All vasodilator drugs were discontinued 12 hours before laboratory testing. Flow-mediated dilation (FMD) of the brachial (bFMD) and femoral (fFMD) arteries to assess endothelial-dependent reactivity was performed using high-resolution ultrasound imaging (ATL HDI 3000; Advanced Technologies Laboratories, Bothell, WA, USA) equipped with a 10.5MHz transducer before and after EECP treatment. Briefly, for bFMD, resting baseline end diastolic brachial diameters and blood velocity were obtained with the transducer placed 3–5 cm above the antecubital fossa. Thereafter, measurements were made for 30 seconds prior to and for 3 minutes following reactive hyperemia produced by inflating a BP cuff placed on the upper forearm for 5 minutes at 200 mmHg followed by a rapid deflation. Continuous ultrasound images were recorded digitally using Pinnacle Studio Plus 10 (Pinnacle Systems, Mountain View, CA, USA). Brachial artery diameters were determined during end-diastole via Vascular Research Tools (Medical Imaging Applications LLC, Coralville, IA, USA) by measuring the distance between the near and far wall of the intima using the automated edge detection software for video analysis. Peak brachial FMD was expressed as a percentage increase from baseline (FMD%). FMD% is influenced by baseline diameter so absolute changes (mm) in diameter were also determined.⁽³⁴⁾ Brachial FMD measurements were also normalized to the mean shear rate calculated during the first 20 seconds following cuff deflation (relative FMD). Similarly, fFMD was evaluated in the right common femoral artery 2 to 3 cm proximal to the bifurcation with occlusion cuff placement distal to the arterial imaging site at 5 cm above the patella. All bFMD and fFMD 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 blind to left ventricular ejection fraction % and stage of EECP treatment.

Graded Exercise Tests

Graded exercise tests were performed before and after EECP therapy. After 15 minutes of seated rest, heart rate and brachial BP measurements were performed in triplicate in the left arm using an automated noninvasive BP cuff (Omron, Inc., Bannockburn, Illinois, USA). An average of the three heart rate and BP measurements were used for values at rest. All subjects performed symptom limited maximum graded exercise tests (SL-GXT) on a treadmill under a cardiologists supervision using a modified Naughton protocol before and after 35 sessions of EECP. Primary measurements included time to angina, total exercise duration, and peak VO_2 . Criteria for termination of the SL-GXT included 2.5 – 3 mm ST

depression, respiratory exchange ratio (RER) > 1, angina = 2.5 – 3 on a 4 point scale, plateau of VO₂, and volitional fatigue.

Statistical Analysis

All statistical analyses were performed using SPSS version 21.0 for Windows (SPSS, Chicago, IL, USA). Continuous variable data are presented as mean ± SD. All data were tested for normal distribution with the Shapiro-Wilk test for normality. An alpha level of $P < 0.05$ was required for statistical significance. Repeated measures analysis of variance (ANOVA) was used to evaluate the continuous primary dependent variables; peripheral blood pressure, bFMD, fFMD, peak VO₂, peak time to angina, and total exercise time; and the secondary dependent variables, NOx, 6-keto-PGF_{1α}; the serum biomarkers, subject characteristics, and all other data. Pearson correlations were performed to examine relationships between the continuous primary and secondary dependent variables. Within group repeated measures ANOVA's was performed for each variable to analyze the timepoint mean differences from baseline for each group and to determine within group timepoint significance. Further, Tukey post hoc analysis was performed to determine between group differences by utilizing the within subject timepoint effect mean square error and test of between subject effect mean square error derived from the primary repeated measures ANOVA.

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Figure 1.

Data are absolute values from within group repeated measures ANOVA and Tukey post hoc analysis of between-group and between-timepoint differences in absolute values. Absolute values for brachial artery flow mediated dilation (bFMD) are presented: (A) bFMD absolute diameter change (mm); (B) bFMD percent change (%); (C) bFMD normalized to shear rate (s^{-1}); and (D) brachial artery resting diameter (mm) before and after 35 sessions of EECF.

* $P < 0.05$ vs. pretreatment values. Data are expressed as mean $\pm$ SEM.

Figure 2.

Data are absolute values from within group repeated measures ANOVA and Tukey post hoc analysis of between-group and between-timepoint differences in absolute values. Absolute values for femoral artery flow mediated dilation (fFMD) are presented: (A) fFMD absolute diameter change (mm); (B) fFMD percent change (%) (C) femoral artery resting diameter (mm) before and after 35 sessions of EECP. * $P < 0.05$ vs. pretreatment values. Data are expressed as mean $\pm$ SEM.

Figure 3.

Data are absolute values from within group repeated measures ANOVA and Tukey post hoc analysis of between-group and between-timepoint differences in absolute values. Absolute values for plasma nitrate/nitrites (NOx) and 6-keto prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) are presented. (A) NOx (μmol/L); (B) ET-1 (pg/ml) before and after 35 sessions of EECF.

* $P < 0.05$ vs. pretreatment values. Data are expressed as mean $\pm$ SEM.

Figure 4. Two-tailed Pearson correlations between relative brachial artery flow mediated dilation (bFMD), nitric oxide bioavailability (NOx), and exercise duration after EECF treatment.

Table 1

Resting patient descriptive characteristics and metabolic profile.

	CAD (n = 15; 12 male, 3 female)		LVD (n = 9; 7 male, 2 female)	
	Before	After	Before	After
Age, y	65.1 ± 10.4	-	63.6 ± 7.5	-
Height, cm	173 ± 9.5	-	171 ± 7.2	-
Weight, cm	92.1 ± 17.8	92.3 ± 18.4	97.5 ± 13.5	96.8 ± 13.4
BMI, kg/m ²	30.5 ± 4.2	30.6 ± 4.3	33.3 ± 4.2	33.0 ± 4.2
EF, %	53.3 ± 6.6 [†]	-	34.5 ± 4.2 [†]	-
HR, bpm	57.8 ± 8.3	60.8 ± 10.3	61.2 ± 5.2	61.0 ± 7.1
SBP, mmHg	137 ± 18.8	130 ± 13.5 [*]	131 ± 22.9	122 ± 18.3 [*]
DBP, mmHg	75 ± 6.6	74 ± 7.0	76 ± 9.8	72 ± 9.9
Glucose, mg/dl	125 ± 26	119 ± 32	130 ± 27	121 ± 22
Triglycerides, mg/dl	149 ± 54	168 ± 80	152 ± 48	176 ± 71
LDL, mg/dl	69.5 ± 33.4	67.6 ± 28.8	67.5 ± 23.9	71.3 ± 24.8
HDL, mg/dl	40.1 ± 10.3	37.9 ± 11.9	37.6 ± 9.5	38.3 ± 10.7
CCS	3.17 ± 0.37	1.17 ± 0.40 ^{**}	3.14 ± 0.41	1.29 ± 0.43 ^{**}

Values are mean ± SD. Significant values are reported from between-group and between-timepoint repeated measures analysis of variance and Tukey post hoc analysis. CAD indicates coronary artery disease patients with normal left ventricular function; LVD, coronary artery disease patients with left ventricular dysfunction; BMI, body mass index; EF, ejection fraction; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CCS, Canadian Cardiovascular Society angina classification.

^{*} $P < 0.05$ vs. pretreatment values,

^{**} $P < 0.01$ vs. pretreatment values,

[†] $P < 0.01$ between groups at same timepoint.

Table 2

Baseline patient cardiac intervention history and drug regimens.

	CAD (n = 15) (12 male, 3 female)	LVD (n = 9) (7 male, 2 female)
Prior CABG	10 (67)	7 (78)
Prior PTCA	10 (67)	6 (67)
Prior Myocardial Infarction	8 (53)	5 (56)
Multivessel CAD	14 (93)	8 (89)
Diabetes Mellitus	8 (53)	5 (56)
Hypertension	13 (87)	7 (78)
Hyperlipidemia	13 (87)	8 (89)
Lipid-lowering drug	13 (87)	8 (89)
β-blocker	12 (80)	8 (89)
Calcium channel blocker	5 (33)	2 (22)
Long-lasting nitrates	12 (80)	7 (78)
ACE inhibition or ARB	13 (87)	7 (78)
Insulin	3 (20)	2 (22)

Values are presented as the number of patients per group and the percentage within each group (in parentheses). There were no significant differences ($P>0.05$) in baseline characteristics, drug regimens, and cardiac intervention history between CAD and LVD groups at baseline. CAD indicates coronary artery disease with normal left ventricular function; LVD, left ventricular dysfunction; CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty; ACE, angiotensin-converting enzyme; and ARB, angiotensin receptor blocker.

Table 3

Results from symptom-limited graded exercise tests (SL-GXT).

	CAD (n = 15; 12 male, 3 female)		LVD (n = 9; 7 male, 2 female)	
	Before	After	Before	After
Peak VO ₂ (ml/kg/min)	18.2 ± 4.6 [†]	24.8 ± 5.6 ^{*,†}	14.3 ± 4.6 [†]	17.3 ± 5.9 ^{*,†}
Peak Exercise Duration (s)	615 ± 186 [†]	813 ± 223 ^{*,†}	345 ± 191	528 ± 242 ^{*,†}
Peak Time to Angina (s)	421 ± 172	686 ± 193 ^{**}	228 ± 156	359 ± 167 [*]
Peak Angina Rating	2.8 ± 0.8	1.7 ± 1.2 [*]	2.3 ± 1.0	1.5 ± 1.3 [*]
Peak Heart Rate (bpm)	112 ± 11.6	116 ± 16.2	113 ± 14.3	118 ± 17.4
Rating of Perceived Exertion	16.2 ± 1.8	16.9 ± 1.7	16.8 ± 2.1	17.0 ± 1.9
Respiratory Exchange Ratio	0.98 ± 0.08	1.06 ± 0.07	0.96 ± 0.09	1.01 ± 0.06

Values are expressed as mean ± SD. Significant values are reported from between-group and between-timepoint repeated measures analysis of variance and Tukey post hoc analysis. CAD indicates coronary artery disease with normal left ventricular function; LVD, left ventricular dysfunction; BMI, body mass index; EF, ejection fraction; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

^{*} $P < 0.05$ vs. pretreatment values,

^{**} $P < 0.01$ vs. pretreatment values,

[†] $P < 0.05$ between groups at same timepoint.