

Effect of External Counterpulsation on Plasma Nitric Oxide and Endothelin-1 Levels

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Enhanced external counterpulsation (EECP) significantly augments diastolic blood flow and has been postulated to improve endothelial function by increased shear stress. We examined the effects of EECP on plasma nitric oxide and endothelin-1 (ET-1) levels. Plasma nitrate and nitrite (NOx) and ET-1 levels were measured serially in 13 patients with coronary artery disease who received 1-hour daily treatments of EECP over 6 weeks. During the course of EECP therapy, plasma NOx progressively increased and plasma ET-1 progressively decreased. After 36 hours of EECP, there was a $62 \pm 17\%$ increase in plasma NOx compared with baseline (43.6 ± 4.3 vs 27.1 ± 2.6 $\mu\text{mol/L}$, $p < 0.0001$) and a $36 \pm 8\%$ decrease in plasma ET-1 (76.7 ± 9.5 vs 119.5 ± 8.5 pg/L , $p < 0.0001$). At 3 months after completion of EECP, NOx remained $12 \pm 11\%$ above baseline ($p = 0.002$), and ET-1 remained $11 \pm 10\%$ below baseline ($p = 0.0068$). Our data provides neurohormonal evidence to support the hypothesis that EECP improves endothelial function. © 2006 Elsevier Inc. All rights reserved. (Am J Cardiol 2006;98:28–30)

Enhanced external counterpulsation (EECP) is a noninvasive treatment for patients with symptomatic coronary disease and has been shown to decrease angina and extend time to exercise-induced ischemia.^{1,2} The mechanisms responsible for these clinical benefits observed from EECP remain poorly understood. EECP significantly augments diastolic blood flow and increases coronary artery flow,³ thereby increasing endothelial shear stress.⁴ Because shear stress is a potent stimulus for the release of endothelium-derived nitric oxide and modulates release of the vasoconstrictor endothelin-1 (ET-1),^{4,5} it has been postulated that EECP may favorably affect endothelial function. This study evaluated the effect of EECP on plasma nitric oxide and ET-1 levels.

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This study enrolled patients with symptomatic coronary artery disease who were referred for EECP. The study was conducted at the First Affiliated Hospital at Sun-Yat Sen University (Guangzhou, People's Republic of China). Inclusion criteria were age ≥ 18 years and a diagnosis of coronary artery disease by ≥ 1 of the following characteristics: (1) angiographically documented $>70\%$ stenosis of a major epicardial coronary artery and/or $>50\%$ left main coronary artery stenosis or (2) typical symptoms of angina and ischemia documented by exercise electrocardiography

or single-photon emission computed tomography. Patients were excluded for uncontrolled hypertension ($>160/100$ mm Hg), unstable angina, severe aortic insufficiency, atrial fibrillation, frequent premature ventricular complexes (>10 per minute), congestive heart failure of New York Heart Association class $\geq \text{III}$, or severe peripheral vascular disease. All patients gave written informed consent before enrollment and the protocol was approved by the institutional review board at the First Affiliated Hospital.

All subjects were treated with 36 hours of EECP divided into 1-hour daily treatments for 6 days/week over 6 weeks. The EECP device (TS2 Vasomedical, Westbury, New York) is composed of an air compressor, a computer module, a set of cuffs, and a treatment table. For each treatment, cuffs were wrapped around the calves and lower and upper thighs (including the buttocks) of the patient. Cuffs were connected by air hoses to the air-compressor unit. The EECP device inflates the cuffs with air and then deflates them in a sequence that is synchronized to the patient's cardiac cycle. Pressure is applied sequentially from the calves to the buttocks, starting in early diastole. At the end of diastole, the compressed air is released rapidly from the cuffs to remove the externally applied pressure. EECP was performed at external cuff pressures of 0.35 to 0.40 kg/cm^2 . Medications and exercise regimens remained unchanged during the study.

Whole peripheral blood samples were drawn through a 25-gauge needle and collected in tubes coated with ethylenediaminetetraacetic acid immediately before EECP, after 1 hour, 12, 24, and 36 hours of EECP, and at 1 and 3 months after completion of EECP. All blood samples were obtained at the same time of the day for each patient. After an immediate centrifugation at 5,000 rpm for 10 minutes at 4°C , serum was collected and stored at -70°C until use.

Serum samples from each subject were assayed for ET-1

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Table 1

Percent change in plasma endothelin-1 and nitrate and nitrite levels in response to enhanced external counterpulsation

Time	NOx ($\mu\text{mol/L}$)	p Value	ET-1 (pg/L)	p Value
Before EECP	27.1 \pm 2.6		120 \pm 9	
1 h of EECP	+14 \pm 16%	0.014	-6 \pm 6%	0.0065
12 h of EECP	+30 \pm 10%	<0.0001	-17 \pm 7%	<0.0001
24 h of EECP	+48 \pm 15%	<0.0001	-25 \pm 6%	<0.0001
36 h of EECP	+62 \pm 17%	<0.0001	-36 \pm 8%	<0.0001
1 mo after EECP	+29 \pm 16%	<0.0001	-20 \pm 13%	0.0004
3 mo after EECP	+13 \pm 11%	0.002	-11 \pm 10%	0.0068

All comparisons are made with baseline (before EECP) values.

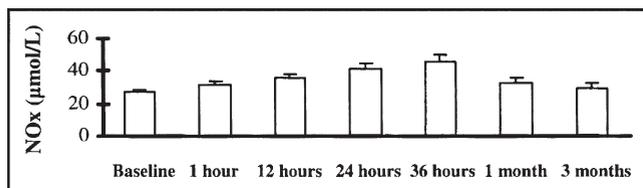


Figure 1. Plasma NOx levels before, during, and at 1 and 3 months after EECP treatment.

by a commercially available enzyme-linked immunosorbent kit (Endothelin-1 Enzyme Immunoassay Kit, Cayman Chemical, Ann Arbor, Michigan). The intra- and interassay coefficients of variance were <10%.⁶

Nitric oxide is a labile compound with a short half life, rapidly converted to the stable end-products nitrate and nitrite (NOx) in oxygenated aqueous solutions, and subsequently excreted into urine. Enzymatic reduction for conversion of nitric oxide to NOx was done by nicotinamide adenine dinucleotide phosphate-dependent enzyme nitrate reductase, followed by spectrophotometric analysis of total NOx as an indicator of recent nitric oxide production in biologic samples *in vivo*.⁷ Serum (250 μL) was incubated at room temperature with 250 μL of substrate buffer in the presence of nitrate reductase for 45 minutes. Excess reduced nicotinamide adenine dinucleotide phosphate was oxidized by continuation of the incubation with 5 μg (1 ml) of lactate dehydrogenase, 0.2 mmol/L (120 μL) of pyruvate (Sigma, St. Louis, Missouri), and 79 ml of water. Reacted samples were treated with 500 μL of trichloroacetic acid (20%) and centrifuged for 15 minutes at 8,000 rpm, and absorbance at 548 nm was compared with that of sodium NOx as a standard.

Continuous variable data are presented as mean \pm SD. Repeated measures generalized estimating equations using an exchangeable correlation that specified the Huber-White-sandwich (robust) estimator of variance, followed by paired 2-sided Student's *t* test, was used to detect differences in plasma NOx or ET-1 levels between baseline (before EECP) and sequential time points. For all tests, a 2-sided *p*-value <0.05 was considered statistically significant. Statistical analyses were performed with STATA 9.1 (STATA Corp., College Station, Texas).

All 13 patients who were enrolled completed the study protocol and constituted the study cohort described in this

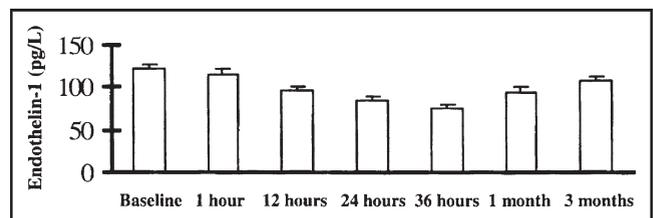


Figure 2. Plasma ET-1 levels before, during, and at 1 and 3 months after EECP treatment.

report. The mean age of the study cohort was 57.6 \pm 9.8 years. Five subjects (38%) were women.

During the course of EECP therapy, plasma NOx levels progressively increased and plasma ET-1 levels progressively decreased (Table 1 and Figures 1 to 2). After the first hour of EECP, there was a 13.8 \pm 15.7% increase in plasma NOx ($p = 0.014$) and a 5.6 \pm 8.5% decrease in plasma ET-1 ($p = 0.0065$). After 36 hours of EECP, there was a 61.6 \pm 17.3% increase in NOx level compared with baseline (2.01 \pm 0.20 vs 1.25 \pm 0.12 mg/L, $p < 0.0001$) and a 36.3 \pm 7.5% decrease in ET-1 (76.7 \pm 9.5 vs 119.5 \pm 8.5 pg/L, $p < 0.0001$).

One month after EECP, the NOx level remained 29.1 \pm 15.5% higher than that at baseline (1.59 \pm 0.15 vs 1.25 \pm 0.12 mg/L, $p < 0.0001$), and the ET-1 level remained 19.9 \pm 12.5% lower than that at baseline (96.0 \pm 11.0 vs 119.5 \pm 8.5 pg/L, $p = 0.0004$). Three months after EECP, plasma NOx remained 12.5 \pm 10.8% above baseline ($p = 0.002$) and plasma ET-1 remained 10.6 \pm 10.2% below baseline ($p = 0.0068$).

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In this prospective study that evaluated the effects of EECP on plasma NOx and ET-1 levels, we have demonstrated that EECP progressively increases NOx and decreases ET-1 levels over the course of therapy. These significant changes are sustained 3 months after EECP therapy.

In a study of 11 patients, Masuda et al⁶ reported a significant increase in plasma NOx levels (compared with baseline) 1 month after completion of a 35-hour course of EECP therapy. Our data are consistent with this observation. Masuda et al⁶ also reported no change in plasma NOx level 1 day or 1 week after completion of EECP. Data were not collected during the course of EECP treatment. In comparison, we found a significant increase in plasma NOx immediately after the first hour of EECP therapy, with progressive increases in NOx during the course of treatment.

This apparent discrepancy may be explained by the short half life of nitric oxide (seconds) and the different mechanisms by which EECP may increase plasma NOx levels. Early increase in plasma NOx may reflect immediate nitric oxide release from EECP-induced increases in diastolic blood flow and venous return. Diastolic blood flow has been shown *in vivo* to increase NOx levels in a pulsatile manner from the beating heart.⁷ Local myocardial concentrations of

NOx are higher in diastole than in systole.⁷ Further, in vivo NOx synthesis has been shown to be directly related to ventricular loading conditions, with rapid increases in NOx occurring over several cardiac cycles in response to abrupt increases in ventricular preload.⁷ Sustained increase in plasma NOx may result from endothelial shear stress causing upregulation in NOx synthase expression, which can result in a delayed but persistent increase in plasma NOx levels.⁸ Early increases in plasma NOx from EECP may be short-lived and disappear as soon as 1 day after EECP therapy, as data by Masuda et al⁶ suggest. However, the dose-related, progressive increase in plasma NOx during EECP therapy observed in our study favors a sustained effect on plasma NOx levels.

By demonstrating that EECP markedly increases plasma NOx and decreases plasma ET-1, our study provides neurohormonal evidence to support the hypothesis that EECP improves endothelial function. This hypothesis is further supported by previous studies that demonstrated by endothelium-dependent brachial artery indexes (reactive hyperemia/peripheral arterial tonometry and flow-mediated dilation) that EECP improves endothelial function in patients with symptomatic coronary artery disease, even after the first hour of therapy.^{8,9} Increases in plasma NOx and resultant potent vasodilation may significantly contribute to improved myocardial perfusion and coronary flow reserve that has been previously documented after EECP.⁶ The time course of improved brachial artery indexes closely follows the pattern of improved and sustained increases in NOx that we observed in our study.

Limitations of this study include a small sample, use of indirect measurements of endothelial function, and lack of a control group for co-treatment interventions, such as medications, lifestyle modifications, or exercise regimen changes, which can influence plasma ET-1 or NOx levels. Patients

were encouraged to continue their diet, pharmacologic, and exercise regimens at a constant level during EECP treatment and the 3 months after therapy.

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