Enhanced External Counterpulsation Inhibits Intimal Hyperplasia by Modifying Shear Stress–Responsive Gene Expression in Hypercholesterolemic Pigs

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Background—Enhanced external counterpulsation (EECP) is a circulation assist device that may improve endothelial dysfunction by increasing shear stress. Chronic exposure of vascular endothelial cells and vascular smooth muscle cells to relatively high physiological shear stress has antiproliferative and vasoprotective effects. The present study hypothesizes that EECP inhibits intimal hyperplasia and atherogenesis by modifying shear stress–responsive gene expression.

Methods and Results—Thirty-five male pigs were randomly assigned to 3 groups: high-cholesterol diet (n = 11), high-cholesterol diet plus EECP (n = 17), and usual diet (control; n = 7). The coronary arteries and aortas were collected for histopathological study and immunohistochemical and Western blot analysis. The peak diastolic arterial wall shear stress during EECP increased significantly compared with before EECP (49.62 ± 10.71 versus 23.92 ± 7.28 dyne/cm²; P < 0.001). Intimal hyperplasia was observed in the coronary arteries of the high-cholesterol diet group, whereas in animals receiving EECP, the intima-to-media area ratio was significantly decreased by 41.59% (21.27 ± 10.00% versus 36.41 ± 16.69%; P = 0.008). Hypercholesterolemia attenuated the protein expression of endothelial NO synthase and enhanced the phosphorylation of extracellular signal-regulated kinases 1/2. EECP treatment alleviated these adverse changes.

Conclusions—EECP reduces hypercholesterolemia-induced endothelial damage, arrests vascular smooth muscle cell proliferation and migration, decreases proliferating cell nuclear antigen proliferative index, suppresses extracellular matrix formation, and eventually inhibits intimal hyperplasia and the development of atherosclerosis by increasing the arterial wall shear stress, which in turn activates the endothelial NO synthase/NO pathway and probably suppresses extracellular signal-regulated kinases 1/2 overactivation. (Circulation. 2007;116:526-534.)

Key Words: atherosclerosis ■ endothelium ■ hypercholesterolemia ■ nitric oxide synthase ■ remodeling

It is currently recognized that hemodynamic forces regulate multiple cellular functions in the cardiovascular system. Vascular endothelial cells (VECs) are constantly exposed to and sensitive to fluid shear stress, the dragging force generated by blood flow. Fluid shear stress modulates endothelial structure and function and is one of the major determinants of arterial tone, vascular remodeling, and atherogenesis. It has been shown that atherosclerotic lesions preferentially develop at certain locations such as bifurcations or side branches that are regions of disturbed flow or low shear stress. On the other hand, laminar flow generating physiological high shear stress can confer anti-inflammatory, antithrombotic, and antiproliferative properties.
creases exercise capacity in the majority of patients undergoing treatment.\textsuperscript{4,5} Growing evidence suggests that improvement in endothelial function represents an important mechanism for the clinical benefits of EECP.\textsuperscript{6,7} However, the exact molecular mechanism remains elusive, and no atherosclerotic animal model other than the use of an acute EECP treatment protocol has been reported.

The present study was designed to investigate the effect of EECP on vascular endothelial function, vascular remodeling, and atherogenesis, as well as VEC gene expression in a porcine experimental model of hypercholesterolemia. We hypothesized that EECP could increase shear stress in vivo, improve both structure and function of the vascular endothelium, inhibit intimal hyperplasia, and therefore arrest atherogenesis through modulating shear stress–responsive gene expression such as upregulating vascular endothelial NO synthase (eNOS) expression and probably downregulating expression such as upregulating vascular endothelial NO synthase (eNOS) expression and probably downregulating extracellular signal-regulated kinases 1/2 (ERK1/2) activity.

**Methods**

**Animal Model**

Thirty-five male Yorkshire (male) and Landrance (female) crossbred pigs (Swine Plant of South China University, Guangzhou, China) with an average weight of 7.4 kg were randomized to 3 groups (7.6±0.7 kg in the control group; 7.2±0.8 kg in the high-cholesterol diet [CHOL] group; and 7.4±0.8 kg in the high-cholesterol diet + EECP [CHOL+EECP] group). All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Seven pigs were fed a usual diet (control group) over the course of 15 weeks. Twenty-eight pigs were fed a high-cholesterol atherogenic diet containing 4% cholesterol, 10% yolk powder, 8% lard, and 1.2% salts for 15 weeks. After 8 weeks of high-cholesterol feeding, while still on the atherogenic diet, 17 hypercholesterolemic pigs were subjected to EECP intervention (CHOL+EECP group). All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Seven pigs were fed a usual diet (control group) over the course of 15 weeks.

**EECP Protocol**

Pigs in the CHOL+EECP cohort received a total of 34±2 hours of EECP treatment, 2-hour sessions every other day over a 7-week period, imitating the standard clinical EECP protocol. With the use of a clinical system (Shuangshan EECP-MCI, Guangzhou, China), EECP was performed by laying the pigs on their left side and wrapping 2 sets of cuffs modified to closely fit the low extremities and the hips of the pigs. The cuffs were sequentially inflated with compressed air from distal to proximal in early diastole and rapidly deflated right before systole. The pressure applied to the cuffs was set at 0.035 to 0.040 mPa/cm\textsuperscript{2}. Effective hemodynamic changes of EECP were demonstrated by achieving a diastolic-to-systolic ratio >1.2 with the use of the plethysmographic technique.

**Western Blot Analysis**

The arch of the aorta was snap-frozen and homogenized in T-PER tissue protein extraction reagent (Pierce Chemical Co, Rockford, Ill) at 0°C to 4°C. The protein concentration was determined with a protein assay system according to the manufacturer (Bio-Rad Dc; Bio-Rad Laboratories, Hercules, Calif). Equal amounts of total protein, 30 μg each, were subjected to electrophoresis on SDS-PAGE with the use of a 6% gel for eNOS and a 10% gel for phospho-ERK1/2. The separated proteins were electrophoretically transferred to Hybond-PVDF membranes (Amersham Pharmacia Bio). After blocking, the membranes were incubated with eNOS antibody (at 1:200 dilution) or phospho-ERK1/2 antibody (at 1:1000 dilution; Cell Signaling Technology, Danvers, Mass), rabbit polyclonal phospho-ERK antibody (at 1:100 dilution; Dako, Glostrup, Denmark), or mouse monoclonal smooth muscle α-actin antibody (at 1:100 dilution; Boster Biological Technology, Inc, Wuhan, China). The proliferation index was defined as the percentage of PCNA-positive cell number against the total nucleated cell number. The percentage of the intimal area occupied by the α-actin–positive area was assessed. Controls in the absence of primary antibodies were also performed.

**Immunohistochemical Study**

Serial paraffin-embedded sections of LADs were used for immunostaining according to the streptavidin-biotin complex or the Elivision immunohistochemical technique.\textsuperscript{10} They were incubated with rabbit polyclonal phospho-ERK antibody (at 1:100 dilution; Cell Signaling Technology, Danvers, Mass), rabbit polyclonal eNOS antibody (at 1:70 dilution; Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), mouse anti–proliferating cell nuclear antigen (PCNA) antibody (at 1:100 dilution; Dako, Glostrup, Denmark), or mouse monoclonal smooth muscle α-actin antibody (at 1:100 dilution; Boster Biological Technology, Inc, Wuhan, China). The proliferation index was defined as the percentage of PCNA-positive cell number against the total nucleated cell number. The percentage of the intimal area occupied by the α-actin–positive area was assessed. Controls in the absence of primary antibodies were also performed.

**Morphological Evaluation**

At the termination of the experiment, a 1-cm segment of the left anterior descending coronary artery (LAD), 0.5 cm distal to the bifurcation of the left main coronary artery, was harvested and fixed and then cut into 3 segments: proximal, middle, and distal. The paraffin-embedded sections were sectioned into 4-μm-thick cross sections and stained with hematoxylin–eosin or Gomori’s aldehyde–fuchsin elastic stains. Parameters were measured at ×40 magnification with the use of a Zeiss-KONTRON IBAS 2.5 Automatic Image Analysis System (Zeiss, Munich, Germany). The intimal-to-medial ratio and wall-to-lumen ratio were calculated for evaluation of vascular remodeling. To quantify collagen deposition in the coronary artery wall, van Gieson–stained sections were scanned to assess the percentage of the intimal and partial medial area occupied by collagen. For each LAD, at least 3 sections from different segments were analyzed. Segments of the LADs were treated for scanning electron microscopy (Hitachi S520). Morphological evaluation and Western blot band densitometry and immunohistochemical analyses were performed by technicians who were blinded to the group assignment.

**Hemodynamic Measurements**

At week 15, Doppler flow examination was performed with the use of a color Doppler ultrasound system (ATL-HDI-5000, Phillips Com America) equipped with an ECG-triggered instrument and a 5- to 10-MHz multifrequency high-resolution linear probe, as previously described.\textsuperscript{8} For the calculation of wall shear stress, internal diameter and blood flow velocity in the right brachial artery were measured just before and during EECP treatment. Peak diastolic velocity was recorded as the mean of 3 cardiac cycles.

The intensity of wall shear stress (τ) was calculated according to the formula τ (dyne/cm\textsuperscript{2}) = 4ηV/ID, where η is the viscosity expressed in poise, V is the blood flow velocity expressed in centimeters per second, and ID is the internal diameter expressed in centimeters.

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Results

Characteristics of Cholesterol-Fed EECP Pigs
At the end of the study at week 15, the body weight of the CHOL+EECP group was slightly but not significantly less than that of the CHOL group ($P>0.05$) (Table). The atherogenic diet resulted in a 4.79-fold increase in serum cholesterol level and 6.66-fold increase in low-density lipoprotein level in the CHOL group. The cholesterol levels of the CHOL+EECP group were slightly lower than those of the CHOL group, but this difference was not statistically significant ($P>0.05$).

Hemodynamic Effects of EECP
At week 15, the blood viscosity of the CHOL+EECP group was $3.8\pm0.4$ mPa (0.038±0.004 poise). The peak flow velocity recorded in the cardiac diastolic phase by Doppler ultrasonic system during EECP in the right brachial artery was significantly elevated compared with the pre-EECP condition (24.62±4.74 versus 59.48±13.60; $P<0.001$). The internal diameter of the right brachial artery was not significantly changed during EECP (1.65±0.42 versus 1.68±0.44 mm; $P>0.05$). The peak diastolic arterial wall shear stress during EECP was increased by >2-fold compared with the pre-EECP condition (23.92±7.28 versus 49.62±10.71 dyne/cm²; $P<0.001$), as shown in Figure 1.

Morphological Studies

Scanning Electron Microscopic Analysis
The luminal surface of coronary arteries of the CHOL group was covered with a considerable amount of adherent cells, and the VECs were also irregularly arrayed and markedly desquamated, representing remarkable damage of the endothelium (Figure 2B). In contrast, in the CHOL+EECP group, there was less cellular adherence and less VECs desquamated (Figure 2B).

### Morphological Studies

#### Scanning Electron Microscopic Analysis

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### Statistical Analysis

Data are presented as mean±SD unless indicated otherwise. When homogeneity of variance was confirmed by the Levene variance homogeneity test, statistical comparison among 3 groups was performed by 1-way ANOVA. If the $F$ test results were $<0.05$, post hoc comparisons were performed with the Bonferroni test. A value of $P<0.05$ was considered statistically significant. When variables violated the assumptions for variance homogeneity, the Kruskal-Wallis ranking test was alternatively used, with $P<0.05$ representing significant differences. Post hoc comparisons were performed by the Mann-Whitney test for 2 independent samples recording exact 2-tailed significance. To avoid an accumulation of errors due to multiple comparisons, the levels of significance were adjusted by Holm’s method. It was used, for example, in the results presented in Figure 5: $P<0.017$ was considered statistically significant for comparison of the CHOL group versus the control group, $P<0.025$ for the CHOL group versus the CHOL+EECP group, and $P<0.05$ for the control group versus the CHOL+EECP group.

When multiple time point measurements were taken over time (cholesterol and low-density lipoprotein measurements), repeated-measures analysis was performed with the general linear models general factorial ANOVA, followed by the aforementioned methods. SPSS 13.0 software was used for all statistical calculations.

### Group Characteristics and Morphological Analysis of Porcine LADs at Week 15

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control $(n=7)$</th>
<th>CHOL $(n=11)$</th>
<th>CHOL+EECP $(n=17)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>71.50±4.80</td>
<td>72.71±6.35</td>
<td>70.38±2.32</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>1.88±0.32</td>
<td>9.01±2.89*</td>
<td>6.98±2.85*</td>
</tr>
<tr>
<td>Low-density lipoprotein, mmol/L</td>
<td>0.91±0.27</td>
<td>6.06±2.47*</td>
<td>4.72±2.57*</td>
</tr>
<tr>
<td>SMC fraction, %</td>
<td>15.13±13.84</td>
<td>53.11±16.61*</td>
<td>24.54±17.45†</td>
</tr>
<tr>
<td>PCNA index, %</td>
<td>42.01±18.65</td>
<td>75.07±12.47*</td>
<td>51.72±12.62†</td>
</tr>
<tr>
<td>Collagen fraction, %</td>
<td>12.35±6.85</td>
<td>26.55±10.05*</td>
<td>12.03±2.94†</td>
</tr>
<tr>
<td>Intimal area, $\times10^4 \mu m^2$</td>
<td>11.58±5.67</td>
<td>28.72±14.25*</td>
<td>13.61±6.84†</td>
</tr>
<tr>
<td>Medial area, $\times10^4 \mu m^2$</td>
<td>59.38±14.56</td>
<td>73.92±11.29</td>
<td>65.28±11.64</td>
</tr>
<tr>
<td>Intima-to-media area ratio, %</td>
<td>19.70±9.01</td>
<td>36.41±16.69</td>
<td>21.27±10.00†</td>
</tr>
<tr>
<td>Luminal diameter, $\mu m$</td>
<td>355.43±123.47</td>
<td>339.50±42.88</td>
<td>355.42±44.87</td>
</tr>
<tr>
<td>Wall thickness (OD), $\mu m$</td>
<td>55.62±6.79</td>
<td>77.84±11.15*</td>
<td>60.39±10.75†</td>
</tr>
<tr>
<td>Wall-to-lumen ratio, %</td>
<td>18.04±8.23</td>
<td>23.95±4.44</td>
<td>17.57±4.70†</td>
</tr>
</tbody>
</table>

Values are mean±SD. SMC indicates smooth muscle cell; OD, outer diameter.

* $P<0.01$ compared with control group.
† $P<0.01$ compared with CHOL group.
tion, and the VECs tended to align in parallel to the direction of blood flow, as shown in Figure 2C.

**Intimal Hyperplasia**
The LAD of the CHOL group showed marked intimal thickening and significant atherosclerotic lesions, as demonstrated in Figure 3B and 3E. Morphometric analysis revealed that the intimal area ($P=0.004<0.025$ versus control group) and wall thickness ($P<0.001$ versus control group) were significantly larger in the CHOL group than in the normal control group, as shown in the Table. In the CHOL+EECP pigs, the intimal area was significantly smaller ($P=0.001<0.017$ versus CHOL) and the LAD wall thickness was significantly thinner ($P=0.001<0.05$ versus CHOL) than in the CHOL group, as demonstrated in Figure 3C and 3F. Meanwhile, the differences of medial area, medial thickness, and area within the internal elastic lamina and external elastic lamina among the 3 groups did not reach statistical significance ($P=0.05$). EECP treatment resulted in a significantly reduced intima-to-media area ratio ($P=0.008<0.017$ versus CHOL) and wall-to-lumen ratio ($P=0.004<0.05$ versus CHOL), showing an antihyperplastic effect (Table).

**Figure 2.** Scanning electron micrographic characteristics of the porcine LADs (magnification ×500). A, Representative micrograph of normal control group. B, Representative micrograph of CHOL group. The luminal surface was covered with many adherent cells; serious endothelial cell disarrangement and desquamation were observed. C, Representative micrograph of CHOL+EECP group. Less cellular adherence could be observed. VECs aligned parallel to the direction of blood flow.

**Figure 3.** Effect of EECP on the histomorphology and intimal hyperplasia of LADs of hypercholesterolemic pigs. A, B and C, Representative photomicrographs (magnification ×40) of elastic-stained (dark purple) LAD. D, E and F, Representative photomicrographs (magnification ×400) of LAD sections stained with hematoxylin-eosin (HE). A and D, Control group. B and E, CHOL group. C and F, CHOL+EECP group. G, Effect of EECP on intima-media thickness ratio of porcine LAD. Significant intimal thickening could be observed in the CHOL group. EECP treatment resulted in diminished intimal hyperplasia in the CHOL+EECP group. Arrows indicate internal elastic lamina. Values are mean±SD. *$P=0.007<0.05$; †$P=0.002<0.05$ vs CHOL group, 1-way ANOVA and Bonferroni test.
Vascular Smooth Muscle Cell Proliferation
In hypercholesterolemic pigs, vascular smooth muscle cells (VSMCs) proliferated and migrated from the media into the intima through the broken and disarranged internal elastic lamina (Figure 4B). VSMC volume fraction, as represented by the percentage of intima area occupied by smooth muscle cells in the intima,\(^{11}\) was significantly elevated in the CHOL group compared with the normal control group (\(P<0.005\) versus control), as shown in the Table. EECP treatment reduced the smooth muscle cell fraction by 53.79% compared with the CHOL group (\(P<0.005\) versus CHOL group) indicated that the proliferative changes induced by hypercholesterolemia were significantly restored by EECP intervention (Figure 4F).

Collagen Remodeling
Accompanying thinning and breakage of the internal elastic lamina, elastic fibrin proliferation and disarrangement could be observed in the hyperplastic intima of the LAD (Figure 3B). There also appeared to be a marked increase in collagen accumulation in the hyperplastic intima and partial media (\(P<0.001\) versus control group) (Figure 4H). The percentage of collagen-positive area in the CHOL+EECP group was significantly lower than that in the CHOL group (\(P<0.001\)). These results indicate that the proliferation of vascular connective tissue and extracellular matrix caused by hypercholesterolemia was inhibited by EECP treatment, as shown in the Table and Figure 4I.

Proliferation Index
As shown in Figure 4, PCNA-positive cells (mainly VECs and VSMCs) were detected in the intima and the medial layer of the LADs. The PCNA-positive index was significantly greater in the coronary arteries of the CHOL group than in those of the normal control group (\(P<0.001\) versus control), as shown in Figure 4E and the Table. The decreased number of PCNA-positive cells in the coronary arteries of the CHOL+EECP group (\(P=0.005<0.05\) versus CHOL group) indicated that the proliferative changes induced by hypercholesterolemia were significantly restored by EECP intervention (Figure 4F).
Effect of EECP on Level of eNOS Protein Expression

Micrographs of porcine LADs with eNOS antibody immunohistochemical staining are shown in Figure 5. The eNOS protein was localized mainly in VECs. In the CHOL group, a remarkably weaker eNOS expression was observed (Figure 5B). In the CHOL+EECP group, the reduction of eNOS expression was not significant (Figure 5C).

Western blotting showed that the amount of eNOS protein was significantly decreased in the CHOL group compared with the control group ($P=0.009<0.017$). The eNOS protein level in the CHOL+EECP group was 3.16 times that of the CHOL group ($P=0.023<0.025$ versus CHOL). These results suggest that EECP treatment significantly elevates eNOS protein expression and alleviates the severe inhibition of eNOS expression induced by hypercholesterolemia. The changes observed with Western blot also correlated strongly with immunohistochemistry results.

Effect of EECP on Phospho-ERK1/2 Activity

As shown by Western blot analysis, the ERK1/2 activity (expressed as the ratio of phospho-ERK1/2 to total ERK1/2) was higher in the CHOL group ($P=0.035>0.017$ for ERK1 and $P<0.001$ for ERK2 versus control). In the CHOL+EECP group, the ERK1/2 activity showed a descending tendency ($P=0.036>0.025$ for ERK1 and $P=0.026>0.025$ for ERK2 versus CHOL), as shown in Figure 6E. These results indicated that EECP intervention presumably inhibited the phosphorylation of ERK1/2 induced by hypercholesterolemia. The changes observed with Western blot also correlated strongly with immunohistochemistry results.

Discussion

Wall shear stress is probably the most important local factor able to influence atherogenesis. In large arteries, the magnitude of shear stress is in the range of 10 to 70 dyne/cm$^2$. Arterial level shear stress ($>15$ dyne/cm$^2$) induces endothelial quiescence and an atheroprotective gene expression profile, whereas low shear stress ($<4$ dyne/cm$^2$), which is prevalent at atherosclerosis-prone sites, stimulates an atherogenic phenotype. Shear stress regulates endothelial structure and function by regulating the expression of mechanosensitive genes. VECs subjected to a long duration of laminar shear stress at the relatively high levels have a lower rate of DNA synthesis than those under static conditions. In animals with vessel grafts or stented vessels, increased local shear stress induces regression of intimal hyper-
plasia. In humans, it has also been demonstrated that augmentation of wall shear stress inhibits neointimal hyperplasia after stent implantation.\(^{15,16}\)

The systolic deflation/diastolic inflation sequence of EECP leads to systolic unloading and diastolic augmentation, resulting in increased blood flow in a pulsatile manner. In the present study, the data showed that during EECP, the peak diastolic arterial wall shear stress increased \(>2\)-fold. EECP has the definite effect of elevating arterial wall shear stress in vivo, whereas most drugs have failed to demonstrate such beneficial hemodynamic effects.

Using histopathological and morphological investigations, we have provided evidence in a porcine model that EECP improves hypercholesterolemia-induced endothelial damage, arrests collagen remodeling, and inhibits the proliferation and migration of VSMCs, findings supported by the reduction of the cellular proliferative index. The significant effects of EECP on these main components of atherosclerotic lesions provide support for the hypothesis that EECP can inhibit intimal hyperplasia induced by hypercholesterolemia.

Acute increase in shear stress can cause acute robust NO production, which plays a critical role in vessel relaxation, whereas chronic NO production due to the increased laminar shear stress may serve as an antiatherogenic and anti-inflammatory molecule.\(^{17}\) Being the rate-limiting enzyme essential for NO synthesis, and with shear stress–responsive elements in its gene promoter region, eNOS may serve as a mechansensor coupling NO release to long-term hemodynamic changes.\(^{18}\) Previous studies have shown that a primary defect in the NO synthase/NO pathway can accelerate neointimal formation and impair endothelial function.\(^{19}\) In contrast, strategies aimed at increasing local NO production, such as eNOS gene transfer\(^{20}\) or delivery of NO donor,\(^{21}\) inhibit neointimal thickening after artery injury. Both clinical investigation and animal studies from our laboratory have shown that EECP treatment increased plasma NO levels and eNOS gene expression.\(^{22,23}\) In the present study, our results indicate that hypercholesterolemia markedly decreased eNOS protein level, whereas EECP significantly elevated eNOS protein expression.

It is well known that regular physical exercise improves endothelial function by increasing blood flow and shear stress, which in turn enhances the eNOS/NO pathway.\(^{24}\) In this aspect, the mechanism of EECP is similar to physical exercise. Because most cardiac patients cannot exercise sufficiently to achieve a similar degree of increase in arterial shear stress, EECP can help to provide a vascular protective benefit similar to that of vigorous exercise.

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**Figure 6.** Effect of EECP on hypercholesterolemia-induced activation of ERK1/2. A, B, and C, Representative photomicrographs (magnification \(\times400\)) of immunohistochemical staining against phospho-ERK1/2 (brown staining, nucleus localized) of porcine LADs. The expression of phospho-ERK1/2 was increased remarkably in the CHOL group but inhibited in the CHOL + EECP group. A, Control group. B, CHOL group. C, CHOL + EECP group. D, Representative Western blot bands of phospho-ERK1/2 and total ERK1/2 (after stripping) of porcine aorta. E, Histograms showing the fluorescence intensity of bands expressed as the ratio of phospho/total ERK1/2. The hypercholesterolemia-induced ERK1/2 phosphorylation was inhibited in the CHOL + EECP group. Results are expressed as mean\(\pm\)SEM of 6 independent experiments from 32 porcine protein samples. \(^{#}P<0.035<0.017\) for ERK1 CHOL group vs control group; \(^{*}P<0.001<0.017\) for ERK2 CHOL group vs control group; \(^{1}P<0.036<0.025\) for ERK1 CHOL + EECP group vs CHOL group; \(^{§}P<0.026<0.025\) for ERK2 CHOL + EECP group vs CHOL group; Mann-Whitney test adjusted by Holm’s method.
Inhibition of ERK1/2 phosphorylation has been shown to be critical in NO inhibition of cellular proliferation. NO superoxide and peroxynitrite induced by fluid shear stress appear to function as signaling molecules in flow-dependent activation of mitogen-activated protein kinases. A previous study has documented that exogenous NO mediates both temporal gradient shear-induced activation and steady shear-induced downregulation of phospho-ERK1/2. On the other hand, NOS blockade stimulates protein synthesis by enhancing ERK1/2 phosphorylation in large arteries. As the central element of growth signaling pathway, ERK1/2 is thought to be directly involved in transmitting signals from growth factor receptors to the nucleus to regulate gene transcription and protein synthesis, leading to proliferation, differentiation, or apoptosis. In the present study, ERK1/2 phosphorylation was upregulated in the hypercholesterolemic control pigs, whereas it was downregulated in the EECP group. ERK1/2 phosphorylation was inversely related to eNOS expression, consistent with previous studies. These results suggest that EECP therapy helps VECs to maintain an atheroprotective gene profile and resist the deleterious effects of hypercholesterolemia.

One limitation of the present study is the relatively small number of samples, which may influence the power of statistical tests. ERK activity showed a downregulating tendency in animals receiving EECP compared with hypercholesterolemic controls, but differences did not reach statistical significance because the sample size may have been underpowered. Another limitation refers to the probable systematic bias in Doppler hemodynamic measurements performed by the same ultrasound technician, who was not blinded to the group assignment. In addition, the hemodynamic parameters were measured in a peripheral artery and therefore cannot be used directly to draw any conclusion about shear stress in the coronary circulation. Still another limitation refers to the lack of a control group that used uncoordinated EECP.

In conclusion, the present study demonstrates a correlation between EECP-mediated increased shear stress and the inhibition of intimal hyperplasia, supporting the antiatherogenic effect of chronic exposure to shear stress, and suggests that EECP should be considered a therapeutic strategy for the treatment of atherosclerotic occlusive disease.

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Disclosures
Dr Lawson served on the Speakers’ Bureau of Vasomedical. Dr Hui served as chief technology officer and senior vice president of Vasomedical. The other authors report no conflicts.

References


### CLINICAL PERSPECTIVE

Wall shear stress is probably the most important local factor affecting atherogenesis. Lowered shear stress, which is prevalent at atherosclerosis-prone sites, usually leads to atherosclerosis, whereas physiological shear stress at a relatively high level has an antiproliferative and vasoprotective effect. In the present study, enhanced external counterpulsation (EECP) is used as a means to increase shear stress to examine its effects on hypercholesterolemic-induced intimal hyperplasia. Histopathological, morphological, and molecular biological techniques were used to provide evidence in a porcine model to demonstrate that EECP helps to repair endothelial damage, attenuates collagen remodeling, arrests the proliferation and migration of vascular smooth muscle cells, and eventually inhibits intimal hyperplasia. Clinically, EECP has been shown to increase NO release and improve endothelium-dependent flow-mediated dilation of the brachial artery. Our model takes a further step to study the molecular mechanisms underlying the clinical benefits obtained by EECP. These findings highlight a novel modality to regulate endothelial shear stress–responsive gene expression, suggesting EECP as a useful therapeutic strategy for the prevention and treatment of atherosclerotic disease.
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