In Vivo Gene Transfer of Endothelial Nitric Oxide Synthase to Carotid Arteries From Hypercholesterolemic Rabbits Enhances Endothelium-Dependent Relaxations

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Background and Purpose—Hypercholesterolemia is associated with abnormal endothelium-dependent vasorelaxation due to decreased nitric oxide bioavailability. Our aim was to examine the effect of adenovirus-mediated gene transfer of endothelial nitric oxide synthase (eNOS) to the hypercholesterolemic rabbit carotid artery in vivo. In addition, we examined whether adenovirus-mediated gene transfer was associated with vascular dysfunction.

Methods—Rabbits were fed a 1% cholesterol diet for 4 weeks followed by a 0.5% cholesterol diet for 6 weeks. Vascular reactivity was assessed in nontransduced carotid arteries from chow- and cholesterol-fed animals. In addition, carotid arteries were surgically isolated, and 2 separate doses of adenoviral vectors encoding eNOS or β-galactosidase (AdβGal) on the contralateral side were delivered to the lumen (1×10^10 and 5×10^10 pfu/mL).

Results—Abnormal acetylcholine-mediated endothelium-dependent vasorelaxation was detected in the carotid artery from cholesterol-fed animals, whereas responses to calcium ionophore A23187 and diethylamine NONOate were normal. Vascular reactivity was similar in nontransduced and AdβGal-transduced hypercholesterolemic vessels. In vessels transduced with eNOS, transgene expression was demonstrated by immunostaining in both the endothelium and the adventitia and by Western blot analysis. High-dose but not low-dose eNOS gene transfer enhanced endothelium-dependent relaxation in vessels from cholesterol-fed rabbits.

Conclusions—Adenovirus-mediated gene transfer of eNOS to carotid arteries of cholesterol-fed animals improves endothelium-dependent relaxation when an optimal viral titer is administered. (Stroke. 2000;31:968-975.)

Key Words: gene transfer ■ genetic vector ■ hypercholesterolemia ■ nitric oxide ■ nitric oxide synthase ■ rabbits
target for gene therapy. Another factor to be borne in mind when considering gene therapy approaches to cholesterol-induced abnormal endothelium-dependent relaxation is the possibility of vector-induced toxicity. Adenoviral vectors have been reported to induce vascular dysfunction, which may obviate any beneficial effect of NOS overexpression. Thus, gene therapy approaches to cholesterol-induced abnormal endothelium-dependent relaxation will be complicated by a number of issues, including disease stage, choice of NOS isoform, and potential for vector-induced toxicity. In the current study, we decided to test the effect of eNOS overexpression on cholesterol-induced abnormal endothelium-dependent relaxation, because this isoform is constitutively expressed in the endothelium. In addition, as gene transfer was being accomplished with adenoviral vectors, we examined the potential for vector-induced changes in vascular reactivity.

Materials and Methods

Construction, Propagation, and Purification of Adenoviral Vectors

A recombinant adenovirus encoding the eNOS gene driven by a cytomegalovirus promoter was generated as previously described. Bovine eNOS cDNA was cloned into the pACCMVpLpA vector, a kind gift of Robert Gerard (University of Texas Southwestern Medical Center, Dallas, Tex). The resulting plasmid was linearized and cotransfected with dl309 into 293 cells through calcium phosphate/DNA coprecipitation. Recombinant adenoviral vectors were generated through homologous recombination. Viral plaques were picked and propagated in 293 cells. Viral DNA was enriched through Hirt extraction and screened with restriction mapping and polymerase chain reaction for the presence of eNOS cDNA. Positive plaques underwent 2 further rounds of plaque purification in 293 cells. Virus was purified by double cesium chloride gradient ultracentrifugation and was dialyzed against 10 mmol/L Tris, 1.0 mmol/L MgCl₂, 1.0 mmol/L HEPES, and 10% glycerol for 4 hours at 4°C. Viral titer was obtained from Dr. James Wilson (University of Pennsylvania, Philadelphia) and was dialyzed against 10 mmol/L K₂HPO₄, 1 mmol/L PMSF, and 0.2% SDS—8% polyacrylamide gel. The resolved proteins were transferred to a nitrocellulose membrane on a semidry electroblotting apparatus and was visualized with the ECL Western blotting detection system (Amersham Life Science). Bovine aorta endothelial cell extracts was used for a positive control.

Detection of Plasma Lipids

Blood samples were centrifuged at 2000 rpm for 10 minutes at 4°C, and plasma was stored at −70°C until lipid measurements. Total plasma cholesterol and triglyceride levels were measured according to standard enzymatic techniques.

Histological Analysis

After harvesting, the arterial rings from the chow- and cholesterol-fed animals were fixed in formalin and then embedded in paraffin. Four 5-μm-thick sections at 25-μm intervals were collected on glass slides and stained with hematoxylin and eosin.

Detection of eNOS Expression With Western Blot Analysis

Carotid arteries were isolated and immediately stored in liquid nitrogen. The frozen segments were pulverized and solubilized in lysis buffer (100 mmol/L K₂HPO₄, 1 mmol/L PMSF, and 0.2% Triton X-100). Carotid debris was homogenized on ice and then centrifuged at 4000 rpm for 10 minutes to remove the insoluble pellet, and protein concentration was determined with the bicinchoninic acid assay. Fifty micrograms of protein was loaded onto an SDS—8% polyacrylamide gel. The resolved proteins were transferred to 0.2-μm nitrocellulose membrane on a semidry electrophoretic transfer cell (Bio-Rad) for Western blot analysis. Blots were blocked and incubated with mouse anti-human eNOS monoclonal IgG1 (dilution 1:250) (Transduction Laboratories) for 1 hour at room temperature and, after washing, secondary antibody (anti-mouse IgG, horseradish peroxidase–linked whole antibody [from sheep; dilution 1:1500; Amersham Life Science]). The secondary antibody was visualized with the ECL Western blotting detection system (Amersham Life Science). Bovine aorta endothelial cell extracts were used for a positive control.

Histochemical and Immunohistochemical Analyses of Gene Expression

Rings were fresh frozen in O.C.T. compound (Miles, Inc), and serial 5-μm-thick sections were cut. For histochemical staining of...
β-galactosidase, sections were fixed in 2% paraformaldehyde and 0.4% glutaraldehyde for 15 minutes at 4°C and then rinsed twice with PBS. Sections were stained in a solution of 500 μg/mL 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; Boehringer-Mannheim Biochemicals) for 4 hours at 37°C and then were rinsed in PBS and counterstained with eosin. For immunohistochemical staining of recombinant eNOS, after immersion fixation in acetone (4°C) and drying, the slide was incubated in 0.1% sodium azide/0.3% hydrogen peroxide and then incubated with 5% goat serum/PBS-Tween 20 to block nonspecific protein-binding sites. An eNOS monoclonal antibody (1 μg/mL incubated for 1 hour at room temperature, followed by incubations with biotinylated rabbit anti-mouse F(ab)2 secondary antibody (1:100) and peroxidase-conjugated streptavidin (1:300, incubation time 20 minutes; DAKO) and peroxidase-conjugated streptavidin (1:300, incubation time 20 minutes; DAKO). After a 30-second immersion in 0.1 mol/L sodium acetate buffer (pH 5.2), eNOS immunoreactivity was visualized with 3-amino-9-ethylcarbazole and hematoxylin counterstaining.

**CD31 Staining**

After immersion fixation in acetone (4°C) and drying, the slide was washed 3 times with PBS. After blocking by 10% normal goat serum in 0.3% Triton X-100 for 2 hours, CD31 monoclonal antibody (dilution 1:50; Jackson ImmunoResearch Laboratories Inc) was applied for 60 minutes at room temperature, followed by incubations with biotinylated rabbit anti-mouse F(ab’)2 secondary antibody (1:300, incubation time 20 minutes; DAKO) and peroxidase-conjugated streptavidin (1:300, incubation time 20 minutes; DAKO). After a 30-second immersion in 0.1 mol/L sodium acetate buffer (pH 5.2), CD31 immunoreactivity was visualized with 3-amino-9-ethylcarbazole and hematoxylin counterstaining.

**Analyses of Vascular Reactivity**

Rings (4 mm long) from each carotid artery were used for assessment of vascular reactivity. Rings were suspended in organ chambers filled with 25 mL of gassed (95% O2/5% CO2) modified Krebs-Ringer bicarbonate solution (pH 7.4, temperature 37°C; composition 118.3 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl2, 1.2 mmol/L MgSO4, 1.2 mmol/L KH2PO4, 25.0 mmol/L NaHCO3, 0.026 mmol/L calcium sodium EDTA, and 11.1 mmol/L glucose). The rings were allowed to equilibrate for 1 hour and then stretched to the optimal point on the length-tension curve as determined through repeated exposure to 20 mmol/L KCl. The maximal contraction of each ring was determined with 60 mmol/L KCl. All concentration responses were determined in the presence of indo- methacin (10−6 mol/L) to block any effects mediated by the activation of cyclooxygenase. Acetylcholine (10−8 to 10−6 mol/L) was added cumulatively during a submaximal contraction to phenylephrine. Submaximal contractions were obtained using a 10−6 to 10−7 mol/L concentration of phenylephrine, with care taken to match the contractions in different experimental groups. Concentration responses were determined in the presence of indo- methacin (10−6 mol/L) to block any effects mediated by the activation of cyclooxygenase. Acetylcholine (10−8 to 10−6 mol/L) was added cumulatively during a submaximal contraction to phenylephrine. Submaximal contractions were obtained using a 10−6 to 10−7 mol/L concentration of phenylephrine, with care taken to match the contractions in different experimental groups. Concentration responses were determined in the presence of indo- methacin (10−6 mol/L) to block any effects mediated by the activation of cyclooxygenase. Acetylcholine (10−8 to 10−6 mol/L) was added cumulatively during a submaximal contraction to phenylephrine. Submaximal contractions were obtained using a 10−6 to 10−7 mol/L concentration of phenylephrine, with care taken to match the contractions in different experimental groups. Concentration responses were determined in the presence of indo- methacin (10−6 mol/L) to block any effects mediated by the activation of cyclooxygenase. Acetylcholine (10−8 to 10−6 mol/L) was added cumulatively during a submaximal contraction to phenylephrine. Submaximal contractions were obtained using a 10−6 to 10−7 mol/L concentration of phenylephrine, with care taken to match the contractions in different experimental groups. Concentration responses were determined in the presence of indo- methacin (10−6 mol/L) to block any effects mediated by the activation of cyclooxygenase. Acetylcholine (10−8 to 10−6 mol/L) was added cumulatively during a submaximal contraction to phenylephrine. Submaximal contractions were obtained using a 10−6 to 10−7 mol/L concentration of phenylephrine, with care taken to match the contractions in different experimental groups. Concentration responses were determined in the presence of indo- methacin (10−6 mol/L) to block any effects mediated by the activation of cyclooxygenase. Acetylcholine (10−8 to 10−6 mol/L) was added cumulatively during a submaximal contraction to phenylephrine. Submaximal contractions were obtained using a 10−6 to 10−7 mol/L concentration of phenylephrine, with care taken to match the contractions in different experimental groups.

**Serum Lipid Levels**

The average plasma cholesterol level in the chow-fed group was 29.2±2.0 mg/dL. In contrast, cholesterol levels were significantly increased in the cholesterol-fed group (1686.0±148.5 mg/dL, P<0.0001). Triglyceride levels were also significantly increased in the cholesterol-fed animals (293.5±29.8 versus 91.1±11.2 mg/dL, P<0.0001). HDL-cholesterol levels were similar in both groups (17.4±2.0 versus 20.5±1.7 mg/dL for cholesterol-fed group versus chow-fed group, P=0.2572).

**Histological Analysis**

Vessels from 16 chow- and cholesterol-fed animals were analyzed. Morphology was assessed in 4 sections of each vessel cut at 25-μm intervals. The vessel segment examined was chosen at random in each case. Intimal lesions were not observed in any of the examined sections.

**Histochemical Localization of β-Galactosidase Expression**

Localization of β-Galactosidase expression was assessed in the low-dose experiment. Arteries transduced with AdβGal at a concentration of 1×1010 pu/mL and harvested 4 days later showed transgene expression in the endothelium and adventitia as confirmed with X-Gal staining. In contrast, there was no staining in the AdeNOS-transduced arteries (Figure 1).

**Detection of eNOS Expression With Western Blot Analysis**

eNOS protein was documented in carotid segments from cholesterol-fed animals after adenovirus-mediated gene trans-
Effects of Hypercholesterolemia on Vascular Reactivity

Initially, we examined vasodilatation in carotid rings from nontransduced chow- and cholesterol-fed animals. Contractions to phenylephrine did not differ significantly between both groups. Vasodilatation in response to acetylcholine obtained during submaximal contractions in response to phenylephrine were significantly impaired in carotid artery segments obtained from hypercholesterolemic rabbits (Figure 4A). In contrast, vasodilatation in response to DEA NONOate or calcium ionophore A23187 was similar in vessels from normocholesterolemic and hypercholesterolemic animals (Figures 4B and 4C).

Effects of In Vivo Gene Transfer of β-Galactosidase on Vascular Reactivity

Carotid artery segments were analyzed from nontransduced cholesterol-fed and AdβGal-transduced cholesterol-fed animals. These vessel segments were obtained from different animals. Vasodilatation in response to acetylcholine obtained during contractions in response to phenylephrine was similar in cholesterol-fed animals and those transduced with AdβGal at both doses (Figure 5). The EC50 value was similar in cholesterol-fed, nontransduced, and AdβGal-transduced vessels at both doses (7.29±0.26 versus 7.30±0.33 versus 7.17±0.13, P=NS). Thus, there was no evidence of vector-induced impaired endothelium-dependent relaxation in this study.

Effects of In Vivo Gene Transfer of eNOS on Vascular Reactivity

We next sought to examine the effect of eNOS gene transfer on cholesterol-induced impaired endothelium-dependent relaxation. Low-dose eNOS gene transfer did not significantly improve impaired endothelium-dependent relaxation in these animals (Figure 6). The EC50 value was similar for cholesterol-fed and low-dose eNOS-transduced vessels (7.29±0.26 versus 7.40±0.45, P=NS). In contrast, relaxation to acetylcholine obtained during submaximal contractions to phenylephrine was significantly enhanced in the high-dose AdeNOS-transduced arteries (Figure 6). The high-dose AdeNOS-induced enhancement was demonstrated by a shift in EC50 value between cholesterol-fed and high-dose eNOS-transduced vessels (7.29±0.26 versus 7.59±0.30, P<0.05).

Discussion

In the present study, eNOS was overexpressed in the hypercholesterolemic rabbit carotid artery with adenovirus-mediated gene transfer. The luminal administration of an adenoviral vector encoding either β-galactosidase or eNOS resulted in endothelial and adventitial transgene expression. Cholesterol feeding for 10 weeks was associated with abnormal receptor-mediated endothelium-dependent vasodilatation. eNOS gene transfer with an adenoviral titer of 5x10^10 pfu/mL improved this abnormality. In contrast, when 1x10^10 pfu/mL AdeNOS was delivered to the vessel lumen, no improvement was observed. In addition, adenovirus-mediated gene transfer to the hypercholesterolemic rabbit carotid artery did not induce impaired endothelium-dependent relaxation at 4 days after transduction.

Hypercholesterolemia is associated with abnormalities of endothelium-dependent relaxation in humans and animals. This has been extensively studied in the cholesterol-fed New Zealand White rabbit. Most literature in this model, however, focuses on the aorta. Fewer studies examine the effect of hypercholesterolemia on endothelium-dependent relaxation in other vascular beds. Abnormal endothelium-dependent relaxation in the aorta and other vascular beds is a well-known feature of this model. In the present study, we examined the effects of hypercholesterolemia on carotid artery function using a well-established model of cholesterol feeding in rabbits. We sought to determine whether overexpression of eNOS in the carotid artery could improve endothelium-dependent relaxation in response to acetylcholine.

Localization of eNOS Expression

To investigate the distribution of NOS expression, immunohistochemistry was performed on frozen tissue sections. Transgene expression was detected in the endothelium and adventitia of the low-dose AdeNOS-transduced arteries. In contrast, there was no staining in AdβGal-transduced arteries (Figure 3). Endothelial integrity was confirmed with CD31 staining (data not shown).

Figure 2. Protein expression assessed with Western blotting in carotid rings after adenovirus-mediated gene transfer of eNOS (AdeNOS; lanes 1, 3, and 5) or β-galactosidase (AdβGal; lanes 2, 4 and 6) (top: A, low dose; B, high dose). Densitometric analysis of eNOS Western blotting (bottom). Data represent mean value of 3 different experiments. eNOS expression was significantly increased in eNOS compared with β-galactosidase–transduced vessels at both doses of vector (P<0.05).
relaxation to acetylcholine in the carotid artery has been reported after 4 and 8 weeks of 1% cholesterol feeding. The response to ionophore and sodium nitroprusside was normal in that study. Laight et al also demonstrated impaired endothelium-dependent relaxation to acetylcholine in the rabbit carotid artery after 8 to 10 weeks of a 1% cholesterol diet. Only 1 dose of acetylcholine was studied, and the response to calcium ionophore A23187 and NO donor was not examined. In contrast, in another report, acetylcholine-induced relaxations remained normal in the hypercholesterolemic rabbit carotid artery. In the current study, animals were fed a high-cholesterol diet for 10 weeks, resulting in severe hypercholesterolemia. Carotid artery atherosclerosis was not detected in these animals. In agreement with the first 2 studies noted earlier, abnormal endothelium-dependent relaxation was demonstrated. In addition to assessment of the response to acetylcholine, we examined the response to calcium ionophore A23187 and NO donor. The finding of impaired relaxation to acetylcholine in the presence of a normal response to calcium ionophore A23187 and NO donor suggests that the abnormality detected in our animal model was due to either a receptor problem or receptor uncoupling. In addition, the normal response to ionophore and NO donor suggests that at this early stage of the disease, substrate availability is not rate limiting and that cofactor levels and NOS expression are not altered.

Adenovirus vectors have been shown to efficiently transduce the vascular wall in vivo. In the current study, we demonstrated gene transfer to the hypercholesterolemic rabbit carotid artery. Of interest, transgene expression was documented in the endothelium and the adventitia of the transduced vessels. Presumably, the latter resulted from movement of vector from the lumen to the adventitia via the vasa vasorum. This was observed in both AdβGal- and AdeNOS-transduced vessels. Thus, in the current model, in which the luminal administration of the adenoviral vector to an isolated segment of carotid artery was used, the effect of overexpression of eNOS in both endothelium and adventitia is examined. The specific cell type expressing the transgene in the adventitia was not examined in this protocol; however, we have previously demonstrated eNOS expression in fibroblasts associated with caveolin after adventitial delivery of an adenoviral vector encoding eNOS. The pattern of staining observed in the present study was similar to our previous report and suggests that the transgene is expressed in adventitial fibroblasts, although it is possible that endothelium of the vasa vasorum is also a site of recombinant eNOS expression.

Adenovirus-mediated gene transfer to the vessel wall has been shown to result in abnormal vascular morphology and function. Newman et al reported the development of intimal hyperplasia 21 days after the delivery of 2×10^10 pfu/mL concentration of an adenoviral vector to the normal rabbit femoral artery. Vasomotor studies were not performed. Lafont et al reported vasomotor dysfunction early after the exposure of normal rabbit arteries to an adenoviral vector. In

![Figure 3. Immunohistochemical localization of eNOS in rabbit carotid artery 4 days after transduction with low-dose AdβGal or AdeNOS vector. There was no positive staining in AdβGal-transduced artery (A and C). Positive immunostaining is seen in endothelium and adventitia in AdeNOS-transduced arteries (B and D). Original magnification ×40 (A and B) and ×100 (C and D).]
that report, 4×10^{10} pfu/mL was delivered to an isolated segment of rabbit ear or femoral artery. The contractile responses to potassium and phenylephrine were reduced in vessels exposed to the adenoviral vector. In addition, relaxation in response to acetylcholine and calcium ionophore A23187 was abolished. Recently, adenovirus-mediated vascular dysfunction was reported to be dose dependent. Macrophage infiltration of baboon hypercholesterolemic vessels after adenovirus-mediated gene transfer has been demonstrated.24 In contrast to the findings of Lafont et al,11 we previously delivered an adenoviral vector encoding β-galactosidase (1×10^{10} pfu/mL) to the rabbit carotid artery via the lumen and adventitia13,14; 4 days later, there was no evidence of vessel wall inflammation or abnormalities of vascular function. Likewise, in the current study, there was no difference in vascular reactivity between nontransduced vessels from hypercholesterolemic animals and AdβGal-transduced vessels at both doses studied. The differences in these results may be due to viral dose and different animal models. In our experience, however, in both the chow- and cholesterol-fed rabbit, adenovirus-mediated gene transfer to the carotid artery is not associated with vascular dysfunction 4 days after transduction with viral doses of 1×10^{10} or 5×10^{10}
pfu/mL. (100 μL). Higher doses and later time points may well be associated with vascular inflammation and abnormal vascular reactivity.

Two reports described improved endothelium-dependent relaxation after ex vivo gene transfer of eNOS to the atherosclerotic rabbit vasculature. In both of these models, adenovirus-mediated gene transfer was achieved after the vessel was harvested from the animal, and organ chamber studies were performed after 24 hours of ex vivo incubation. The dose of adenoviral vector used was 1×10¹⁰ and 3×10¹⁰ pfu/mL, and 100 μL was delivered. Atherosclerosis was present, and therefore, a more advanced disease stage may have been present in those studies. The effect of in vivo nNOS gene transfer to the hypercholesterolemic rabbit carotid artery was recently described. In that study, rabbits have been present in those studies. The effect of in vivo gene transfer, but the dose of vector administered was critical, because no improvement was observed when a dose of 1×10¹⁰ pfu/mL was used. In contrast, when the dose was increased to 5×10¹⁰ pfu/mL, a significant improvement in vasomotor function was observed. Previously, we demonstrated augmented endothelium-dependent vasorelaxation after the administration of 1×10¹⁰ pfu/mL of AdeNOS to the lumen or the adventitia of the carotid artery of the chow-fed rabbit. Thus, it appears that higher concentrations of AdeNOS are required to alter vascular reactivity in the setting of hypercholesterolemia. The 4-day time point was examined because efficient transgene expression is observed then. It is unlikely that the low dose of AdeNOS would have had a beneficial effect if vascular function were examined at a later time point. However, our results and those of Channon et al suggest that both eNOS and nNOS gene transfer to the carotid artery of cholesterol-fed animals has beneficial effects, but in the case of eNOS, the viral dose that was administered was critical. It is of interest that the effect on vascular reactivity may be considered relatively modest, whereas the Western blot data suggest a marked increase in eNOS expression. This may be due to the fact that NO generated form recombinant eNOS in the setting of hypercholesterolemia may be scavenged by oxygen free radicals, thus limiting the amount of bioavailable NO.

In summary, therefore, high-cholesterol feeding to rabbits for 10 weeks resulted in an abnormality of receptor-mediated endothelium-dependent relaxation, which was improved by adenovirus-mediated gene transfer of eNOS at a dose of 5×10¹⁰ pfu/mL. A lower dose of AdeNOS had no effect. In addition, no evidence of adenovirus-induced vascular dysfunction was observed at either dose. These data demonstrate the critical effect of vector dose in studies with the goal of reversing vascular dysfunction through adenovirus-mediated gene transfer. An adenoviral vector dose that lacks toxicity but demonstrates biological efficacy should be determined in such experiments.

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References

Hypercholesterolemia impairs agonist-induced nitric oxide synthase–dependent relaxation of large blood vessels (see Flavahan and Vanhoutte\(^1\) for review). Mechanisms by which hypercholesterolemia impairs nitric oxide synthase–dependent vasorelaxation have been attributed to abnormal receptor function,\(^2\) a decrease in nitric oxide synthase activity,\(^3\) an increase in the formation of oxygen radicals,\(^4\) and/or a decrease in the availability/utilization of nitric oxide.\(^5,6\) Recent studies have demonstrated a potential use of gene transfer to improve nitric oxide synthase–dependent responses of blood vessels.\(^7–9\) The goal of the present study was to examine the effects of gene transfer of eNOS on cholesterol-induced abnormal nitric oxide synthase–dependent vasorelaxation.

Thus, in the present study, the authors examined nitric oxide synthase–dependent reactivity of the carotid artery in chow- and cholesterol-fed rabbits after adenoviral-mediated gene transfer of eNOS. The authors report that receptor-mediated nitric oxide synthase–dependent relaxation of the carotid artery was impaired in cholesterol-fed rabbits. Injection of a low dose of eNOS gene transfer did not improve impaired nitric oxide synthase–dependent vasorelaxation. In contrast, injection of a high titer of eNOS adenovirus significantly improved acetylcholine-induced vasorelaxation. Further, in vessels transduced with eNOS, there was evidence of transgene expression in both the endothelium and adventitia of the carotid arteries. The results of these studies suggest that impaired vasorelaxation during hypercholesterolemia can be improved by injection of a high concentration of adenovirus gene transfer of eNOS. Thus, care should be taken in studies that examine vascular reactivity after gene transfer in order to assure that an adequate titer of adenovirus is delivered to the blood vessel.

**Editorial Comment**

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**References**

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