Inducible Nitric Oxide Synthase and Atherosclerosis

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Summary: Nitric oxide (NO) synthase induction in vascular smooth muscle cells may play a role in local vascular injury associated with atherosclerosis or postangioplasty restenosis by inhibiting smooth muscle cell proliferation and contraction, as well as by preventing leukocyte and platelet adhesion. The expression of inducible NO synthase is increased in balloon-injured arteries of experimental animals or in human atherosclerotic lesions. Replacement therapy with NO donors or NO synthase gene transfer may improve the clinical course of atherosclerosis or restenosis.

Key words: nitric oxide, vascular smooth muscle, atherosclerosis, restenosis, gene transfer

Introduction

Nitric oxide (NO), the extensively characterized endothelium-derived relaxing factor, is a short-lived free radical. It is synthesized from L-arginine by three isoenzymes expressed either constitutively (neuronal, type I nNOS; endothelial, type II eNOS) or following stimulation by cytokines (inducible, type II iNOS).^{1,2} Inducible NO synthase has been identified in endotoxin- and cytokine-treated macrophages, hepatocytes, and myocardium.^{1,3} Inducible NO synthase activity is also induced in cultured vascular smooth muscles and aortic rings by cytokines and endotoxin.^{4,5} Nitric oxide synthase induction in vascular smooth muscle cells may play a role in local vascular injury associated with atherosclerosis or restenosis after an-

gioplasty by inhibiting smooth muscle cell proliferation and contraction, as well as by preventing leukocyte and platelet adhesion. There have been several review articles on the involvement of endothelial NO synthase in atherosclerotic disease,^{6–9} but the involvement of inducible NO synthase of the vascular smooth muscle has not been fully discussed. In this review, we discuss the involvement of inducible NO synthase in the pathogenesis of atherosclerosis and restenosis and its implications for therapeutic strategies.

Effects of Nitric Oxide on the Vascular Tissue

Vascular Tone

Nitric oxide is an endogenous vasodilator. Cytokines such as interleukin-1 β (IL-1 β), expressed in atherosclerotic lesions, activate the synthesis of inducible NO synthase in vascular smooth muscle cells.¹⁰ French *et al.*¹¹ and Takizawa *et al.*¹² reported that IL-1 β -induced NO inhibited vascular smooth muscle contraction. Moritoki *et al.*¹³ investigated the characteristics of L-arginine-induced nonendothelial NO formation in rat isolated thoracic aorta and found that induction of an inducible NO synthase during prolonged incubation with endotoxin, which accelerated production of NO, caused relaxation of the arteries via cyclic GMP (cGMP). When NO reaches vascular smooth muscle cells, guanylate cyclase is activated to synthesize cGMP, which relaxes vascular smooth muscle through mechanisms including hyperpolarization, inhibition of phosphatidic inositol turnover, and inhibition of contractile elements.¹⁴

Local NO production by vascular smooth muscle cells could represent an important mechanism for the maintenance of arterial patency in the injured artery. Provost *et al.*¹⁵ investigated the effects of the NO donor 3-morpholino-sydnonimine (SIN-1) on the vasomotor response in a porcine model of angioplasty. Carotid arterial injury was produced by balloon dilation in control and treated pigs. The internal diameter of the carotid arteries was similar between the two groups before dilation, but was 40% greater at the site of endothelial injury distally in SIN-1-treated animals compared with controls.

Yan *et al.*¹⁶ also studied the effects of inducible NO synthase on blood flow in balloon catheter-denuded rat carotid arteries as a model for arterial injury and restenosis. Arterial

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injury triggered the expression of inducible NO synthase in the lesion, and inhibition of NO synthase by N^G-nitro-L-arginine methylester (L-NAME) led to a 24% reduction of the blood flow in the injured artery, demonstrating that NO produced by inducible NO synthase restores blood flow.

Cell Proliferation

Excessive vascular smooth muscle proliferation is considered to be a hallmark of atherosclerotic disease. Garg and Hassid¹⁷ observed that growth of vascular smooth muscle cells was inhibited by exogenous NO through activation of soluble guanylate cyclase. Nitric oxide donors such as sodium nitroprusside significantly decreased the proliferation of vascular smooth muscle cells, and 8-bromo-cGMP mimicked the antimitogenic effect of NO donors. Moreover, the inhibition of proliferation was shown to be independent of cell damage. Kariya *et al.*¹⁸ showed that cGMP suppressed the proliferation of vascular smooth muscle cells by inhibiting the progression from G1 into S phase of the cell cycle. However, the antiproliferative effect of NO may not depend solely on guanylate cyclase activation, as Garg and Hassid¹⁹ reported a cGMP-independent antimitogenic mechanism of action of NO. Very recently, Kullo *et al.*²⁰ showed that gene transfer of endothelial NO synthase to vascular smooth muscle cells resulted in the expression of a functional enzyme and inhibition of DNA synthesis and cell proliferation.

Leukocyte Adhesion

Leukocytes including monocytes may play an important role in the pathophysiologic response to arterial injury.²¹ Accumulating evidence suggests that NO influences endothelial cell-leukocyte adhesion.²² Nitric oxide donors inhibit leukocyte adhesion, and the NO synthase inhibitor L-NAME enhances leukocyte adhesion in the microcirculation.^{23, 24} Nitric oxide modulates leukocyte and endothelial adhesion molecules, and thus leukocyte adhesion. We²⁵ and De Caterina *et al.*²⁶ previously observed that NO decreased endothelial intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expression. Kubes *et al.*²⁷ showed that a monoclonal antibody directed against the common β -subunit (CD18) of the leukocyte-adhesion glycoprotein complex prevented leukocyte adherence to vascular endothelium induced by L-NAME.

Platelet Adhesion

Nitric oxide acts as endogenous inhibitor of platelet adhesion and aggregation. The antiplatelet activity of NO is due largely to its interaction with soluble guanylate cyclase, which results in an increased cGMP concentration in the platelets.^{28, 29} The subsequent cGMP-mediated reactions are less clear, but NO inhibits the expression of platelet glycoprotein IIb/IIIa and P-selection.^{30, 31}

Provost *et al.*¹⁵ investigated the effects of the NO donor SIN-1 on platelet adhesion in a porcine model of angioplas-

ty. At the site of arterial injury, platelet adhesion was significantly inhibited by SIN-1. Yan *et al.*¹⁶ studied the involvement of inducible NO synthase expressed in the injured vessel wall on platelet adhesion. Systemic administration of L-NAME resulted in a two- to threefold increase in the adhesion of platelets to the injured lesion, demonstrating that NO produced locally by inducible NO synthase inhibits platelet adhesion. Oslen *et al.*³² also reported that platelet deposition on injured vessels was enhanced by removal of NO with hemoglobin or by inhibition of NO synthase and was prevented by the NO precursor L-arginine.

Expression of Inducible Nitric Oxide Synthase in the Vascular Tissue

Inducible NO synthase activity is induced in cultured vascular smooth muscle cells by endotoxin and cytokines.^{5, 10} Cytokine-induced inducible NO synthase expression is enhanced by cyclic adenosine-monophosphate (cAMP)-elevating agents such as adenosine³³ and adrenomedullin,³⁴ while its expression is inhibited by protein kinase C activating agents such as angiotensin II,³⁵ platelet-derived growth factor,³⁶ and endothelin.³⁷ The expression of these humoral factors has been shown to be increased in atherosclerotic arteries and thus may contribute to atherogenesis via modulation of NO production.

It has been demonstrated that inducible NO synthase is expressed following balloon injury of rat carotid arteries or in human atherosclerotic lesions.^{38, 39} Joly *et al.*⁴⁰ demonstrated that balloon injury induced NO synthase activity in rat carotid arteries, even in the absence of endothelium. Yan *et al.*¹⁶ evaluated the time course of expression of inducible NO synthase in balloon injured rat carotid arteries by in situ hybridization and immunohistochemistry. They found that inducible NO synthase mRNA and protein were expressed in the innermost layer of the media from Day 1 and were subsequently detected in the neointima, whereas no expression was detectable in the uninjured artery. Bosmans *et al.*⁴¹ reported that angioplasty led to a significant induction of nonendothelial NO synthase in rabbit carotid arteries. Also, in humans, Buttery *et al.*³⁹ and Wilcox *et al.*⁴² reported that enhanced inducible NO synthase mRNA and protein were found in atherosclerotic lesions, while they were not detected in normal vessels.

Inducible NO synthase induction in vascular smooth muscle cells after injury may be triggered by cytokines released from infiltrating inflammatory cells such as monocytes. We previously reported that the expression of inducible NO synthase in vascular smooth muscle cells was enhanced via their interaction with monocytes.⁴³ A recent report indicating that shear stress response elements are present in the promoter region of the inducible NO synthase gene⁴⁴ led us to speculate that cytokine-independent mechanisms activated during tissue injury are also involved in inducible NO synthase expression. Further studies are necessary to determine whether cytokines and/or other mechanisms are responsible for inducible NO synthase induction following arterial injury.

Therapeutic Implications

Administration of Nitric Oxide Precursor

It has been shown that long-term administration of L-NAME promotes atherosclerosis in the hypercholesterolemic rabbit aorta,^{45, 46} suggesting that impaired NO generation results in atherogenesis. On the other hand, NO activity persisting after intimal injury may inhibit intimal hyperplasia. Long-term supplementation with the NO precursor L-arginine has been shown to enhance NO production at sites of vascular healing and to reduce intimal hyperplasia in the impaired artery.^{47, 48} McNamara *et al.*⁴⁹ reported that L-arginine administration from 2 days prior to and 2 weeks following catheter-induced injury to the rabbit thoracic aorta attenuated the development of intimal hyperplasia by 39% compared with untreated controls. The effect of L-arginine was inhibited by coadministration of the NO synthase inhibitor, L-NAME, suggesting that the mechanism for this effect is the conversion of L-arginine to NO. Furthermore, administration of L-arginine to hypercholesterolemic rabbits has been shown to reverse defects in NO activity and to reduce the development of proliferative atherosclerotic lesions.⁵⁰

Nitric Oxide Synthase Gene Transfer

Wounds require inducible NO synthase to heal at a normal rate, and wound healing in inducible NO synthase-deficient mice can be reconstituted with inducible NO synthase cDNA, suggesting the possibility of therapeutic benefit for delivering NO synthase by gene therapy in some settings. Such approaches have also been contemplated to reduce postangioplasty restenosis. Tzeng *et al.*⁵¹ reported that in vitro retroviral human inducible NO synthase gene transfer to isolated porcine arterial vessel segments subjected to balloon catheter injury completely abrogated the myointimal hyperplasia induced by the injury. von der Leyen *et al.*⁵² demonstrated that local delivery of a cDNA encoding the endothelial NO synthase resulted in 70% reduction of the neointimal mass in the rat carotid artery.

We⁵³ and others^{48, 54, 55} observed that the expression of recombinant endothelial NO synthase in arteries of experimental animals reduced agonist-evoked contractions and enhanced relaxation. Cable *et al.*⁵⁶ reported that adenovirus-mediated gene transfer of endothelial NO synthase to human saphenous veins resulted in functional transgene expression with increased NO release and enhanced relaxation. This technique may reduce the risk of thrombosis and stenosis in saphenous vein grafts.

Conclusion

Excess NO produced by vascular smooth muscle cells via inducible NO synthase may prevent atherosclerosis and restenosis by inhibiting smooth muscle cell proliferation and contraction, as well as by preventing leukocyte and platelet ad-

hesion. However, NO also has toxic and cytolytic effects, and increased expression of inducible NO synthase may promote the process of atherogenesis by increasing cell death and necrosis. Further studies are needed to establish whether NO replacement therapy with NO donors or NO synthase gene transfer can improve the clinical course of atherosclerosis and restenosis.

References

1. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: Physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991;43: 109–140
2. Nathan C, Xie Q: Regulation of biosynthesis of nitric oxide. *J Biol Chem* 1994;269:13725–13728
3. Shindo T, Ikeda U, Ohkawa F, Takahashi M, Funayama H, Nishinaga M, Kawahara Y, Yokoyama M, Shimada K: Nitric oxide synthesis in rat cardiac myocytes and fibroblasts. *Life Sciences* 1994;55:1101–1108
4. Koide M, Kawahara Y, Tsuda T, Yokoyama M: Cytokine-induced expression of an inducible type of nitric oxide synthase gene in cultured vascular smooth muscle cells. *FEBS Lett* 1993;318:213–217
5. Dinerman JL, Lowenstein CJ, Snyder SH: Molecular mechanisms of nitric oxide regulation. *Circ Res* 1993;73:217–222
6. Tanner FC, Boulanger CM, Lüscher TF: Endothelium-derived nitric oxide, endothelin, and platelet vessel wall interaction: Alterations in hypercholesterolemia and atherosclerosis. *Semin Thromb Hemost* 1993;19:167–175
7. Wennmalm Å: Endothelial nitric oxide and cardiovascular disease. *J Intern Med* 1994;235:317–327
8. Warren JB, Pons F, Brady AJB: Nitric oxide biology: Implications for cardiovascular therapeutics. *Cardiovasc Res* 1994;28:25–30
9. McIntyre M, Dominiczak AF: Nitric oxide and cardiovascular disease. *Postgrad Med J* 1997;73:639–643
10. Ikeda U, Kanbe T, Nakayama I, Kawahara Y, Yokoyama M, Shimada K: Aldosterone inhibits nitric oxide synthesis in rat vascular smooth muscle cells induced by interleukin-1 β . *Eur J Pharmacol* 1995;290:69–73
11. French JF, Lambert LE, Dage RC: Nitric oxide synthase inhibitors interleukin-1 β -induced depression of vascular smooth muscle. *J Pharmacol Exp Ther* 1991;259:260–264
12. Takizawa S, Ozaki H, Karaki H: Interleukin-1 β -induced, nitric oxide-dependent and -independent inhibition of vascular smooth muscle contraction. *Eur J Pharmacol* 1997;330:143–150
13. Moritoki H, Takeuchi S, Hisayama T, Kondoh W: Nitric oxide synthase responsible for L-arginine-induced relaxation of rat aortic rings in vitro may be an inducible type. *Br J Pharmacol* 1992;107: 361–366
14. Karaki H, Sato K, Ozaki H, Murakami K: Effects of sodium nitroprusside on cytosolic calcium level in vascular smooth muscle. *Eur J Pharmacol* 1988;156:259–266
15. Provost P, Tremblay J, Merhi Y: The antiadhesive and antithrombotic effects of the nitric oxide donor SIN-1 are combined with a decreased vasoconstriction in a porcine model of balloon angioplasty. *Arterioscler Thromb Vasc Biol* 1997;17:1806–1812
16. Yan Z, Yokota T, Zhang W, Hansson GK: Expression of inducible nitric oxide synthase inhibits platelet adhesion and restores blood flow in the injured artery. *Circ Res* 1996;79:38–44
17. Garg UC, Hassid A: Nitric oxide-generating vasodilators and 8-bromo-cyclic GMP inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 1989;83: 1774–1777
18. Kariya K, Kawahara Y, Araki S, Fukuzaki H, Takai Y: Anti-proliferative action of cyclic GMP-elevating vasodilators in cultured rabbit aortic smooth muscle cells. *Atherosclerosis* 1989;80: 143–147

19. Garg UC, Hassid A: Nitric oxide-generating vasodilators inhibit mitogenesis and proliferation of BALB/c 3T3 fibroblasts by a cGMP-independent mechanism. *Biochem Biophys Res Commun* 1990;171:474-479
20. Kullo IJ, Schwartz RS, Pompili VJ, Tsutsui M, Milstien S, Fitzpatrick LA, Katusic ZS, O'Brien T: Expression and function of recombinant endothelial NO synthase in coronary artery smooth muscle cells. *Arterioscler Thromb Vasc Biol* 1997;17:2405-2412
21. Merhi Y, Lacoste LL, Lam JYT: Neutrophil implications in platelet deposition and vasoconstriction after deep arterial injury by angioplasty in pigs. *Circulation* 1994;90:997-1002
22. Provost P, Lam JYT, Lacoste L, Merhi Y, Waters D: Endothelium-derived nitric oxide attenuates neutrophil adhesion to endothelium under arterial flow conditions. *Arterioscler Thromb* 1994;14:331-335
23. Bath PMW, Hasall DG, Glaswin AM, Palmer RMJ, Martin JF: Nitric oxide and prostacyclin: Divergence of inhibitory effects on monocyte chemotaxis and adhesion to endothelium in vitro. *Arterioscler Thromb* 1991;11:254-260
24. Siegfried MR, Carey C, Ma XI, Lefer AM: Beneficial effects of SPM-5185, a cysteine-containing NO donor, in myocardial ischemia-reperfusion. *Am J Physiol* 1992;263:H771-H777
25. Takahashi M, Ikeda U, Masuyama J, Funayama H, Kano S, Shimada K: Nitric oxide attenuates adhesion molecule expression in human endothelial cells. *Cytokine* 1996;8:817-821
26. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MAA, Shin WS, Liao JK: Nitric oxide decreases cytokine-induced endothelial activation. *J Clin Invest* 1995;96:60-68
27. Kubes P, Suzuki M, Granger DN: Nitric oxide: An endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 1992;88:4651-4655
28. Nguyen BL, Sitoh M, Ware JA: Interaction of nitric oxide and cGMP with signal transduction in activated platelets. *Am J Physiol* 1991;261:H1043-H1052
29. Radomski MW, Rees DD, Dutra A, Moncada S: S-Nitroso-glutathione inhibits platelet activation in vitro and in vivo. *Br J Pharmacol* 1992;107:745-749
30. Langford EJ, Brown AS, Wainwright RJ, de Belder BA, Thomas MR, Smith RE, Radomski MW, Martin JF, Moncada S: Inhibition of platelet activity by S-nitrosoglutathione during coronary angioplasty. *Lancet* 1994;344:1458-1460
31. Horstrup K, Jablonka B, Honig LP, Just M, Kochsiek K, Walter U: Phosphorylation of focal adhesion vasodilator-stimulated phosphoprotein at Ser 157 in intact human platelets correlates with fibrinogen receptor inhibition. *Eur J Biochem* 1994;225:21-27
32. Oslen SB, Tang DB, Jackson MR, Gomez ER, Ayala B, Alving BM: Enhancement of platelet deposition by cross-linked hemoglobin in a rat carotid endarterectomy model. *Circulation* 1996;93:327-332
33. Ikeda U, Kurosaki K, Ohya K, Shimada K: Adenosine stimulates nitric oxide synthesis in vascular smooth muscle cells. *Cardiovasc Res* 1997;35:168-174
34. Ikeda U, Kanbe T, Shimada K: Adrenomedullin increases inducible nitric oxide synthase in rat vascular smooth muscle cells stimulated with interleukin-1. *Hypertension* 1996;27:1240-1244
35. Nakayama I, Kawahara Y, Tsuda T, Okuda M, Yokoyama M: Angiotensin II inhibits cytokine-stimulated inducible nitric oxide synthase expression in vascular smooth muscle cells. *J Biol Chem* 1994;269:11628-11633
36. Scott-Burden T, Schini VB, Elizondo E, Junquero DC, Vanhoutte PM: Platelet-derived growth factor suppresses and fibroblast growth factor enhances cytokine-induced production of nitric oxide by cultured smooth muscle cells. *Circ Res* 1992;71:1088-1100
37. Ikeda U, Yamamoto K, Maeda Y, Shimpo M, Kanbe T, Shimada K: Endothelin-1 inhibits nitric oxide synthesis in vascular smooth muscle cells. *Hypertension* 1997;24:65-69
38. Hansson GK, Geng Y, Holm J, Hårdhammar P, Wennmalm A, Jennische E: Arterial smooth muscle cells express nitric oxide synthase in response to endothelial injury. *J Exp Med* 1994;180:733-738
39. Buttery LD, Springall DR, Chester AH, Evans TJ, Standfield EN, Parums DV, Yacoub MH, Polak JM: Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. *Lab Invest* 1996;75:77-85
40. Joly GA, Schini VB, Vanhoutte PM: Balloon injury and interleukin-1 β induce nitric oxide synthase activity in rat carotid arteries. *Circ Res* 1992;71:331-338
41. Bosmans JM, Bult H, Vrints CJ, Kockx MM, Herman AG: Balloon angioplasty and induction of non-endothelial nitric oxide synthase in rabbit carotid arteries. *Eur J Pharmacol* 1996;310:163-174
42. Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, Harrison DG, Marsden PA: Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. *Arterioscler Thromb Vasc Biol* 1997;17:2479-2488
43. Ikeda U, Maeda Y, Funayama H, Hojo Y, Ikeda M, Minota S, Kano S, Shimada K: Monocyte-vascular smooth muscle cell interaction enhances nitric oxide production. *Cardiovasc Res* 1998;37:820-825
44. Nunokawa Y, Ishida N, Tanaka S: Promoter analysis of human inducible nitric oxide synthase gene associated with cardiovascular homeostasis. *Biochem Biophys Res Commun* 1994;200:802-807
45. Naruse K, Shimizu K, Muramatsu M, Toki Y, Miyazaki Y, Okumura K, Hashimoto H, Ito T: Long-term inhibition of NO synthesis promotes atherosclerosis in the hypercholesterolemic rabbit thoracic aorta. *Arterioscler Thromb* 1994;14:746-752
46. Cayatte AJ, Palacino JJ, Horten K, Cohen RA: Chronic inhibition of nitric oxide production accelerates neointima formation and impairs endothelial function in hypercholesterolemic rabbits. *Arterioscler Thromb* 1994;14:753-759
47. Tarry WC, Makhoul RG: L-arginine improves endothelium-dependent vasorelaxation and reduces intimal hyperplasia after balloon angioplasty. *Arterioscler Thromb* 1994;14:938-943
48. Ooboshi H, Chu Y, Rios CD, Faraci FM, Davidson BL, Heistad DD: Altered vascular function after adenovirus-mediated overexpression of endothelial nitric oxide synthase. *Am J Physiol* 1997;273:H265-H270
49. McNamara DB, Bedi B, Aurora H, Tena L, Ignarro LJ, Kadowitz PJ, Akers DL: L-arginine inhibits balloon catheter-induced intimal hyperplasia. *Biochem Biophys Res Commun* 1993;193:291-296
50. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME: Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. *J Clin Invest* 1992;90:1168-1172
51. Tzeng E, Shears LL II, Robbins PD, Pitt BR, Geller DA, Watkins SC, Simmons RL, Billiar TR: Vascular gene transfer of the human inducible nitric oxide synthase: Characterization of activity and effects on myointimal hyperplasia. *Mol Med* 1996;2:211-225
52. von der Leyen HE, Gibbons GH, Morishita R, Lewis NP, Zhang L, Nakajima M, Kaneda Y, Cooke JP, Dzau VJ: Gene therapy inhibiting neointimal vascular lesion: In vivo transfer of endothelial cell nitric oxide synthase gene. *Proc Natl Acad Sci USA* 1995;92:1137-1141
53. Maeda Y, Ikeda U, Ishibashi S, Shimpo M, Shimada K, Ozawa K: Transfer of endothelial constitutive nitric oxide synthase gene into vascular structure using adeno-associated virus vectors (abstr). *Jpn Circ J* 1997;61:590-591
54. Cable DG, O'Brien T, Kullo I, Schwartz RS, Schaff HV, Pompili VJ: Expression and function of a recombinant endothelial nitric oxide synthase gene in porcine coronary arteries. *Cardiovasc Res* 1997;35:553-559
55. Kullo IJ, Mozes G, Schwartz RS, Gloviczki P, Crotty TB, Barber DA, Katusic ZS, O'Brien T: Adventitial gene transfer of recombinant endothelial nitric oxide synthase to rabbit carotid arteries alters vascular reactivity. *Circulation* 1997;96:2254-2261
56. Cable DG, O'Brien T, Schaff HV, Pompili VJ: Recombinant endothelial nitric oxide synthase-transduced human saphenous veins: Gene therapy to augment nitric oxide production in bypass conduits. *Circulation* 1997;96:II-173-II-178