

Forum Review

Therapeutic Angiogenesis in Diabetes and Hypercholesterolemia: Influence of Oxidative Stress

Munir Boodhwani¹ and Frank W. Sellke²

Abstract

Despite significant improvements in the medical, percutaneous, and surgical management, numerous patients are first seen with non-revascularizable coronary artery disease (CAD). The growth of new blood vessels to improve myocardial perfusion (*i.e.*, therapeutic angiogenesis) is an attractive treatment option for these patients. However, the successes of angiogenic therapy, observed in preclinical studies, have not been realized in clinical trials. Increasing evidence suggests that this discrepancy between animal and human studies may be due to the nature of the substrate, or the molecular and cellular environment within which the angiogenic agent acts. Antiangiogenic influences, including endothelial dysfunction, hypercholesterolemia, and diabetes, are present in virtually all patients with advanced CAD. Recent studies have better characterized the abnormalities associated with these disease states, providing novel targets for intervention. These substrate-modifying interventions can potentially enhance the response to protein-, gene-, or cell-based angiogenic therapy. In this review, we discuss key aspects of the angiogenic process and the pathophysiologic and molecular mechanisms that contribute to an impaired angiogenic response in the setting of endothelial dysfunction, hypercholesterolemia, and diabetes, with a focus on the role of oxidative stress. Last, we briefly explore substrate modifying agents that have been evaluated in preclinical and clinical studies to improve the angiogenic response. *Antioxid. Redox Signal.* 11, 1945–1959.

Introduction

CARDIOVASCULAR DISEASE not only is the leading cause of death, disability, and health care expenditure in the United States, but also is the leading cause of mortality around the world. The principal cardiovascular disorder responsible for increases in cardiovascular mortality is no longer rheumatic disease, but rather ischemic cardiovascular disease (10). The prevalence of its risk factors (*i.e.*, physical inactivity, obesity, diabetes, hypercholesterolemia and smoking) continues to increase worldwide. It is not surprising, therefore, that despite improvements in the management of these cardiovascular risk factors and advances in percutaneous and surgical revascularization methods, coronary artery disease (CAD) affects more than 13 million people in the United States and is responsible for one of every five deaths (44). In a large number of patients, CAD can be of such a diffuse and severe

nature that repeated attempts at catheter-based interventions and surgical bypass may be unsuccessful at restoring normal myocardial blood flow. Up to 20–37% of patients with ischemic heart disease cannot undergo coronary artery bypass surgery (CABG) or percutaneous coronary intervention (PCI), or they receive incomplete revascularization with these standard revascularization strategies (35, 50, 67, 69, 92). Furthermore, incomplete revascularization has been associated with increased mortality and poorer clinical outcome (32, 55).

The goal of therapeutic angiogenesis, with growth factor- or cell-based therapies, is to restore perfusion to chronically ischemic myocardium without intervening on the epicardial coronary vasculature. Early experiments in myocardial angiogenesis with recombinant growth factors or gene-based delivery led to great enthusiasm about their therapeutic potential. However, subsequent application in phase I to III

¹Division of Cardiac Surgery, University of Ottawa Heart Institute, Ottawa, Canada; and ²Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, Harvard University, Boston, Massachusetts.

TABLE 1. PLACEBO-CONTROLLED CLINICAL TRIALS OF GROWTH-FACTOR THERAPY

Author	Year	N	Growth Factor	Vehicle	Delivery
Laham (58)	1999	24	FGF-2	Protein	IM (surgical)
Unger (113)	2000	25	FGF-2	Protein	IC
Pecher (82)	2000	40	FGF	Protein	IM
Vale (115)	2001	6	VEGF	Plasmid	IM (perc)
Simons (98)	2002	337	FGF-2	Protein	IC
Losordo (60)	2002	19	VEGF	Plasmid	IM (perc)
Grines (37)	2002	79	FGF-4	Adenovirus	IC
Grines (38)	2003	52	FGF-4	Adenovirus	IC
Henry (45)	2003	178	VEGF	Protein	IC + IV
Hedman (43)	2003	103	VEGF	Plasmid + adenovirus	IC
Tio (108)	2004	23	VEGF	Plasmid	IM (perc)
Kastrup (53)	2005	80	VEGF	Plasmid	IM (perc)
Ruel (86)	2008	19	VEGF \pm l-arginine	Plasmid	IM (surgical)
Total		984			

IM, intramyocardial; IC, intracoronary; IV, intravenous; perc, percutaneously delivered; year, year of publication.

clinical studies has demonstrated limited clinical benefit, and therapeutic angiogenesis remains an experimental treatment for patients for whom conventional therapies have failed (Table 1).

The discordance between successful preclinical studies and disappointing clinical trials may be explained by a number of factors (99). First, angiogenesis is a complex process that involves interactions between a number of pro- and anti-angiogenic mediators, the endothelium, and the extracellular matrix. It is therefore not surprising that single-agent growth-factor therapy has not led to large functional improvements in patients. Second, patients with end-stage coronary disease are vastly different from the young and healthy animals in whom preclinical testing is typically conducted. The presence of diabetes, hypercholesterolemia, and endothelial dysfunction can significantly limit the effect of growth factors on the angiogenic response (87, 118). Third, the optimal delivery strategy, one that provides local delivery and prolonged exposure to a sufficient dose of growth factor without causing unwanted effects, remains to be discovered. Last, the lack of sensitive assays of myocardial angiogenesis limits our ability to detect small, subclinical changes that may be occurring in response to growth-factor delivery. Despite these limitations, angiogenesis is a critical process that occurs in all humans and, if appropriately modulated, can provide therapeutic benefit to the large population of patients with ischemic coronary artery disease.

Processes Involved in Blood Vessel Formation

An understanding of the biology of growth factors and their therapeutic potential requires the understanding of the processes involved in new blood vessel formation and has been previously reviewed (21, 22). Vasculogenesis, angiogenesis, and arteriogenesis are three processes that may contribute to the growth of blood vessels (57).

Vasculogenesis is the formation of new vessels from pluripotent stem cells, as seen in embryonic development. Increasing evidence suggests that vasculogenesis may also occur in the adult, as seen in the mobilization of endothelial progenitor cells from bone marrow and the incorporation of these cells into foci of neovascularization.

Angiogenesis refers to the growth of capillaries from enlarged venules that sprout capillary buds, become divided by periendothelial cells (intussusception), or are separated by transendothelial cell bridges (bridging) to form capillaries. The process starts with vasodilation and increased permeability to allow extravasation of proteins that modify the extracellular matrix. This is followed by endothelial cell proliferation and migration and tube formation with endothelial cell differentiation in response to the local tissue environment. Angiogenesis is the manner by which capillaries proliferate in healing wounds, along the border of myocardial infarctions, as well as in neoplasms. Whether these vessels are capable of producing physiologically relevant increases in tissue perfusion is debated.

Arteriogenesis is the process that results in the appearance of arteries possessing a fully developed tunica media by proliferation of preexisting arterioles into true collateral arteries. Smooth muscle cells may differentiate from various cell types, including endothelial cells and bone marrow precursors. Arteriogenesis involves smooth muscle cell growth and proliferation, migration, and differentiation to a contractile phenotype (23). An example of arteriogenesis is the development of angiographically visible collaterals in patients with advanced obstructive coronary or peripheral vascular disease.

Angiogenic Signaling

The formation of new blood vessels involves a complex molecular signaling cascade. A significant number of cytokines involved in this process have been identified, including members of the fibroblast growth factor (FGF) family, the vascular endothelial growth factor (VEGF) family, the platelet-derived growth factor (PDGF) family, and angiopoietins (127). VEGFs and FGFs are the most widely studied and the only ones used for clinical studies.

Vascular endothelial growth factors are a family of heparin-binding glycoproteins shown to act as mitogens for vascular endothelial cells as well as to stimulate endothelial progenitor cell mobilization from the bone marrow (5). The family of VEGF molecules includes VEGF [A-D] as well as placental growth factor (PlGF). These ligands interact with a number of different tyrosine kinase receptors (flt-1, flk-1, and flt-4) (15).

VEGFs are expressed in cardiac myocytes and vascular smooth muscle cells, with increased expression in the setting of vascular injury, acute and chronic ischemia, and hypoxia (109). VEGFs bind to their tyrosine kinase receptor, which activates PI3 kinase, leading to the phosphorylation of Akt (protein kinase B). Phosphorylation of Akt has numerous downstream effects, among which is the phosphorylation and activation of endothelial nitric oxide synthase (eNOS), eventually leading to the production and release of nitric oxide (NO) (110). Downstream effects of VEGFs include vascular permeability, increased endothelial cell growth and survival, and formation of tubular structures (127). The VEGF family of growth factors has been demonstrated to be a crucial component of redox cell signaling that occurs in response to ischemia and reperfusion and provides the stimulus for neovascularization (64, 65).

The FGF family consists of 23 proteins that are classified by their expression pattern, receptor-binding preference, and protein sequence (29, 34). FGF is present in the normal myocardium (24). Its expression is stimulated by hypoxia (9) and hemodynamic stress (89). FGF-2 is a pluripotent molecule and modulates numerous cellular functions in multiple cell types. In the context of angiogenesis, it induces endothelial cell proliferation, survival, and differentiation, and also is involved in the migration of endothelial cells, smooth muscle cells, macrophages, and fibroblasts (29). These effects are mediated through its interaction with the tyrosine kinase receptor FGFR1 (29, 131), leading to the activation of protein kinase C (α and ϵ isoforms) and also involves syndecan-4 as a downstream mediator. Although FGF signaling also involves NO release (100), in contrast to VEGF, a lesser number of studies have tied the angiogenic effects of FGF-2 directly to NO. Additionally, FGF-2 stimulates endothelial cells to produce a variety of proteases, including plasminogen activator and matrix metalloproteinases (27, 120), promoting chemotaxis.

Role of Substrate in Determining Effects of Therapeutic Angiogenesis

As discussed earlier, one of the major reasons for the discordant results between successful animal models and less efficacious clinical studies is the presence of important pathophysiologic changes in patients with end-stage coronary artery disease in whom angiogenic therapy has been attempted. Despite important advances in risk factor management and medical therapy, patients with advanced coronary artery disease have a number of influences that can impair their response to therapeutic angiogenic therapy. Cardiovascular risk factors, such as hypertension, hypercholesterolemia, diabetes, metabolic syndrome, and smoking all have independent effects on vascular function. However, in addition to these independent effects, a common pathway in which this vascular impairment is manifest is the presence of endothelial dysfunction. Increasing awareness of these anti-angiogenic influences in patients with coronary disease has led to the emergence of the notion that the substrate, or the molecular, cellular, and microvascular environment, on which the therapeutic angiogenic agent acts is as important, if not more important, than the agent itself. In the following sections, we discuss the link between endothelial dysfunction, hypercholesterolemia, and diabetes, and the response to therapeutic angiogenesis.

The endothelium, nitric oxide, and angiogenesis

The endothelium is a critical component in the maintenance of normal vascular function and the response to injury. Although many different aspects of endothelial dysfunction exist, in the context of vascular physiology and angiogenesis, it is generally defined as a reduction in the release of nitric oxide in response to a stimulatory agent. This reduction in stimulated NO release can be evaluated by using a number of *in vivo* or *ex vivo* experimental methods. Most commonly, *ex vivo* assessments of arteries or arterioles are performed by using tension or size-based assessment of vascular reactivity.

A strong relation exists between the release of NO and the regulation of blood-vessel growth and development, especially that mediated by the actions of VEGF. Arnal *et al.* (4) showed that proliferating endothelial cells express about sixfold as much eNOS mRNA as do confluent cells. Substance P and VEGF, which both stimulate the release of NO (93, 134), induce new vessel formation *in vivo* in addition to increasing the permeability, migration, and proliferation of postcapillary endothelial cells in tissue culture (70). Bouloumie *et al.* (132–134) demonstrated that VEGF enhances the expression of eNOS in native and cultured endothelial cells, an effect that may be important in the process of VEGF-induced angiogenesis. Inhibitors of NOS suppress angiogenesis, and the proliferative effect of VEGF is decreased in the presence of NOS inhibitors. Uhlmann *et al.* (112) measured the proliferation and migration of choroidal endothelial cells after VEGF stimulation in the presence or absence of *N*^ω-nitro-arginine methyl ester (L-NAME), a NO inhibitor, and found that pretreatment with L-NAME attenuated the VEGF-induced angiogenic response, in direct correlation with a reduction in basal NO release. NO may also play a crucial role in the VEGF-mediated angiogenic response of vascular smooth muscle cells (VSMCs). Jozowicz *et al.* (51) recently examined the effect of exogenous and endogenous NO on the synthesis of VEGF by rat and human VSMCs by exposing cells to exogenous NO donors, or to the genetic augmentation of eNOS or iNOS. NO donors potentiated by twofold the generation of VEGF protein by rat or human VSMCs. Similarly, rat or human VSMCs transiently transfected with plasmid cDNA encoding eNOS or iNOS synthesized up to threefold more VEGF than did those transfected with control plasmid cDNA, an effect that was reversed after treatment with L-NAME, an eNOS inhibitor.

In comparison to VEGF, a lesser number of studies have tied the angiogenic effects of FGF-2 to local NO availability. Still, NO likely acts as an important signal in the angiogenic response to FGF-2 as well, presumably by terminating its proliferative actions and promoting the differentiation of endothelial cells into vascular tubes (6). This role is supported by the work of Muhohara *et al.* (74), who showed that the inhibition of endothelial NOS by L-NAME attenuated endothelial cell migration but not proliferation *in vitro*. These authors also demonstrated that endogenous endothelium-derived NO maintains the functional expression of integrin $\alpha_v\beta_3$, a mediator for endothelial migration, survival, and angiogenesis, suggesting that endothelium-derived NO plays a crucial role in mediating angiogenesis by supporting endothelial cell migration, at least partly *via* an integrin-dependent mechanism. Recently, Sieber *et al.* (96) studied the role of NO in the effects of FGF-2 in a rat model of portal hypertension

secondary to portal vein ligation. These authors used two Teflon rings, filled with collagen I, that were fixed in the mesenteric cavity, with one supplemented with 100 ng of FGF-2. The role of NO was tested in a subset of animals by adding the NO-formation antagonist *N*^ω-nitro-L-arginine (NNA) to drinking water. After 16 days, the rings were explanted and embedded, and the vessels were morphometrically counted. FGF-2 significantly stimulated vessel formation per implant in control rats, but not in rats with portal hypertension, suggesting that endothelial dysfunction and diminished NO availability may have played an inhibitory role on the effects of FGF-2. Interestingly, the numbers of ingrown vessels without FGF-2 stimulation were higher in rats that had portal vein ligation compared with controls. NNA substantially inhibited angiogenesis in both groups, and FGF-2 did not reverse angiogenesis prevented by NNA.

It also has been demonstrated that tube development by growing endothelial cells in three-dimensional gels in response to transforming growth factor- β is dependent on NO and inhibited by antagonists of NOS (81). Moreover, the stimulated synthesis and release of endothelium-derived NO by VEGF and FGF-2 has been shown to be largely regulated by tyrosine kinases (93), further implicating the role of NO in blood vessel formation mediated by these two proteins. Interestingly, activity of the tyrosine kinase Src was also found to protect endothelial cells from apoptosis during VEGF-mediated angiogenesis in chick embryos and mice (31).

Indirect evidence also supports for a crucial role of NO in the angiogenic process. In a rat gastric ulcer model, Ma *et al.* (61) showed that angiogenesis changed in parallel with eNOS expression, and that L-NAME administration significantly reduced both, suggesting that eNOS plays a significant role in gastric ulcer healing. Dewilt *et al.* (28), in a model of renal subcapsular adenocarcinoma in rats, reported that an additional antitumor effect was demonstrated when L-NAME was added to the synergistic combination of melphalan and tumor necrosis factor (tumor sizes decreased from 70 to 100%), suggesting an antiangiogenic role of L-NAME for the treatment of solid tumors in a systemic or regional setting.

Important *in vivo* evidence suggesting that endothelial factors play a major role in mediating the angiogenic response is found in the murine studies of Jang (48) and Duan (30), whose apoE- hypercholesterolemic mice exhibited attenuated collateral vessel formation in response to a FGF-2 disk angiogenesis system and hindlimb ischemia, respectively. This inhibition was, in both studies, fully reversed by the oral administration of L-arginine, which is the substrate for endothelial NO production. Overall, the bulk of evidence therefore suggests that NO production and perhaps other yet unidentified endothelial factors play a significant role in mediating the endogenous as well as the exogenous angiogenic responses, and likely accounts for the attenuated effects of angiogenic therapy observed in humans with end-stage, inoperable CAD who display significant endothelial dysfunction (33, 36, 54, 130).

Effects of hypercholesterolemia

Hypercholesterolemia in patients may occur for a variety of reasons, including dietary intake or abnormalities in lipid and cholesterol metabolism. The effects of diet-induced hypercholesterolemia on endothelial function have been repeatedly

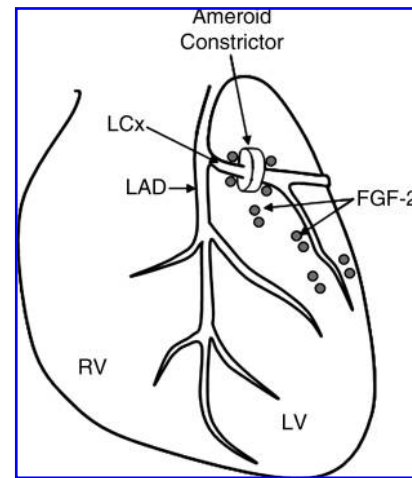


FIG. 1. Schematic showing the commonly used model of circumflex coronary artery ameroid constrictor for the creation of chronic myocardial ischemia as well as perivascular implantation of sustained release FGF-2 heparin alginate beads.

demonstrated in a number of animal models as well as in clinical studies. Cohen *et al.* (25) demonstrated that pigs fed a high-cholesterol diet for as little as 9 weeks had attenuated endothelium-dependent relaxation to serotonin in coronary ring segments placed in an organ chamber, despite the absence of intimal proliferative changes on light or electron microscopy. Similarly, Hasdai *et al.* (40) showed that pigs fed a hypercholesterolemic diet for 10 weeks had reduced vasorelaxation to bradykinin, abnormal responses to the endothelin B-receptor agonist sarafotoxin 6c (41), and impaired arteriolar relaxation to insulin-like growth factor (42). Impairments in smooth muscle function in the setting of hypercholesterolemia have also been demonstrated by Shishido and colleagues (95) in hypercholesterolemic rabbit aortae, in addition to endothelial dysfunction.

To examine the effects of hypercholesterolemia and endothelial dysfunction on angiogenesis, swine fed a diet rich in fat and cholesterol for 13 weeks were subjected to chronic myocardial ischemia by using a circumflex coronary artery ameroid constrictor (Fig. 1). In this model, the endogenous response to chronic myocardial ischemia as well as the exogenous angiogenic response to sustained-release perivascular administration of VEGF and FGF-2 were evaluated (13, 87, 118). All hypercholesterolemic animals demonstrated impaired coronary microvascular relaxation responses to adenosine diphosphate (ADP) and VEGF, suggesting reduced NO availability and endothelial dysfunction. In addition, hypercholesterolemic animals also exhibited impaired relaxation response to the NO donor, sodium nitroprusside, suggesting abnormalities in smooth muscle relaxation. The endogenous response to myocardial ischemia was impaired in the hypercholesterolemic animals, as evidenced by reduced perfusion of the collateral-dependent territory, as well as reduced endothelial cell density in the ischemic circumflex region (13) (Fig. 2). Furthermore, the response to growth factors, VEGF (118) and FGF-2 (87), was impaired compared with that of normocholesterolemic controls. In addition to the

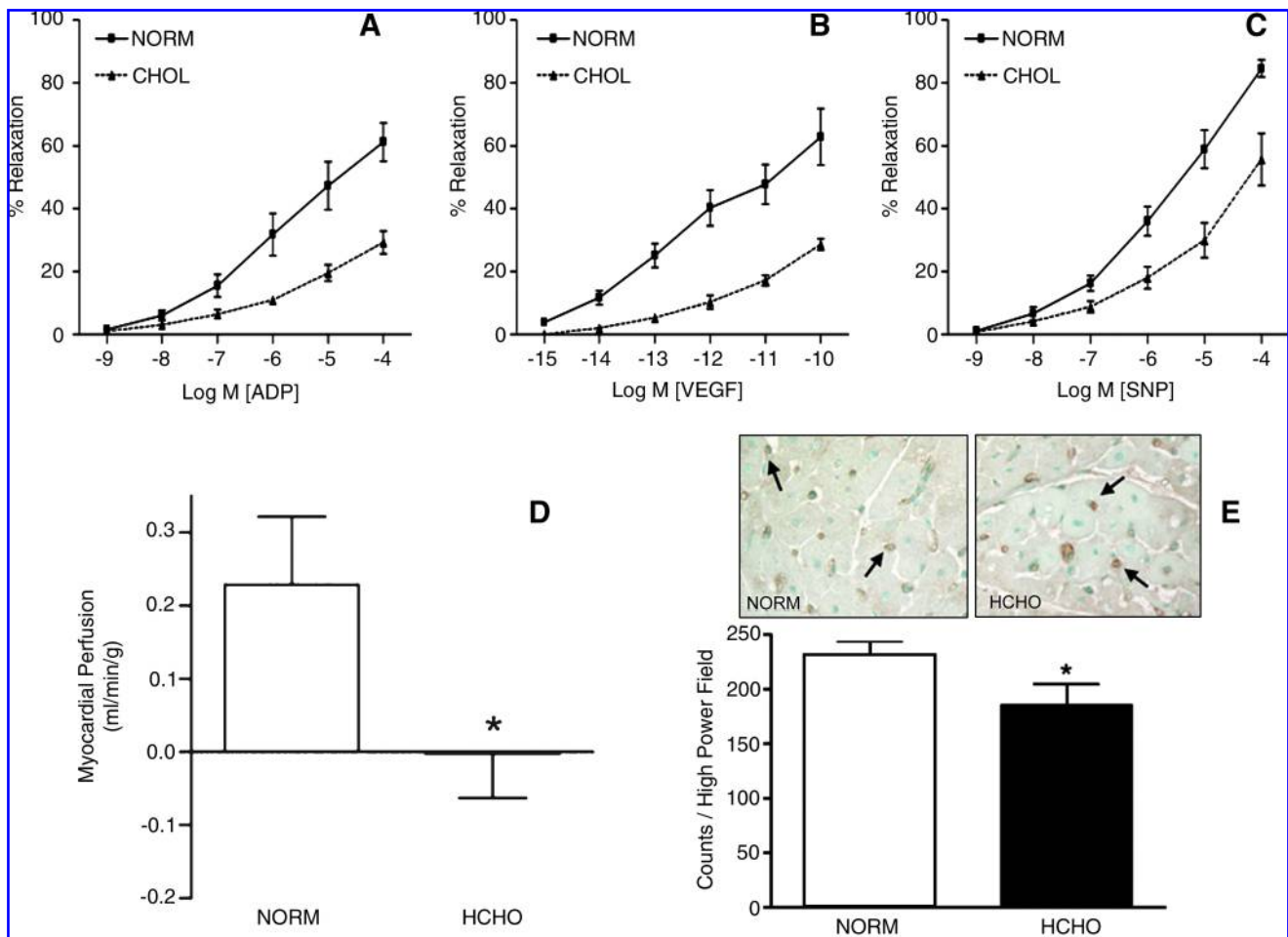


FIG. 2. Summary of functional changes observed in a swine model of hypercholesterolemia and chronic myocardial ischemia. Hypercholesterolemic (HCHO) swine had impaired microvessel relaxation to (A) adenosine diphosphate (ADP), (B) vascular endothelial growth factor (VEGF), and (C) sodium nitroprusside (SNP) compared with normocholesterolemic controls (NORM). HCHO animals had reduced collateral-dependent perfusion (D) and reduced microvessel density (E), indicating impaired angiogenesis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

functional effects, a number of abnormalities at the molecular level were observed in this model.

When examined in a prolonged, stable state 7 weeks after ameroid constrictor placement, the animals demonstrated no significant differences in the protein expression of angiogenic growth factors, their receptors, or any of the downstream mediators, including eNOS. An interesting observation, however, was an increase in oxidation levels of structural proteins in hypercholesterolemic animals, the most prominent of those proteins being the structural protein, actin (51 kDa). This suggests increased oxidative burden in hypercholesterolemic animals (Fig. 3). Reactive oxygen species (ROS) can be generated from numerous sources within the cell, including mitochondria, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase, xanthine oxidase, and eNOS uncoupling (19). In the context of angiogenesis, they are released in response to angiogenic stimuli (for example, ischemia) and play an important role in angiogenic signaling (66). However, ROS also rapidly combine with NO, forming peroxynitrite, and can, therefore, reduce the amount of bioavailable NO (101). Prolonged, excessive production of ROS

can also cause irreversible oxidation of cellular proteins, leading sometimes to altered function (59). The increased burden of oxidative stress in the hypercholesterolemic animal group, as demonstrated by higher levels of oxidized proteins, combined with equivalent levels of eNOS expression in both groups, suggests that the reduced NO bioavailability and resulting endothelial dysfunction in these animals may be due to increased NO degradation, in the presence of ROS, rather than impaired NO synthesis.

Another interesting observation was that the hypercholesterolemic animals demonstrated a significantly higher expression of the antiangiogenic protein, endostatin. Endostatin was originally identified by O'Reilly *et al.* (77) from conditioned medium of a hemangioendothelioma cell line as a highly active and endothelial specific angiogenic inhibitor. It is an endogenous 20-kDa protein that is a C-terminal fragment of collagen XVIII produced by proteolytic cleavage, by a variety of matrix metalloproteinases (MMPs). Endostatin not only may inhibit angiogenesis but also may block migration and proliferation of endothelial cells and increase apoptosis (77). Conversely, one of the proposed physiological effects of

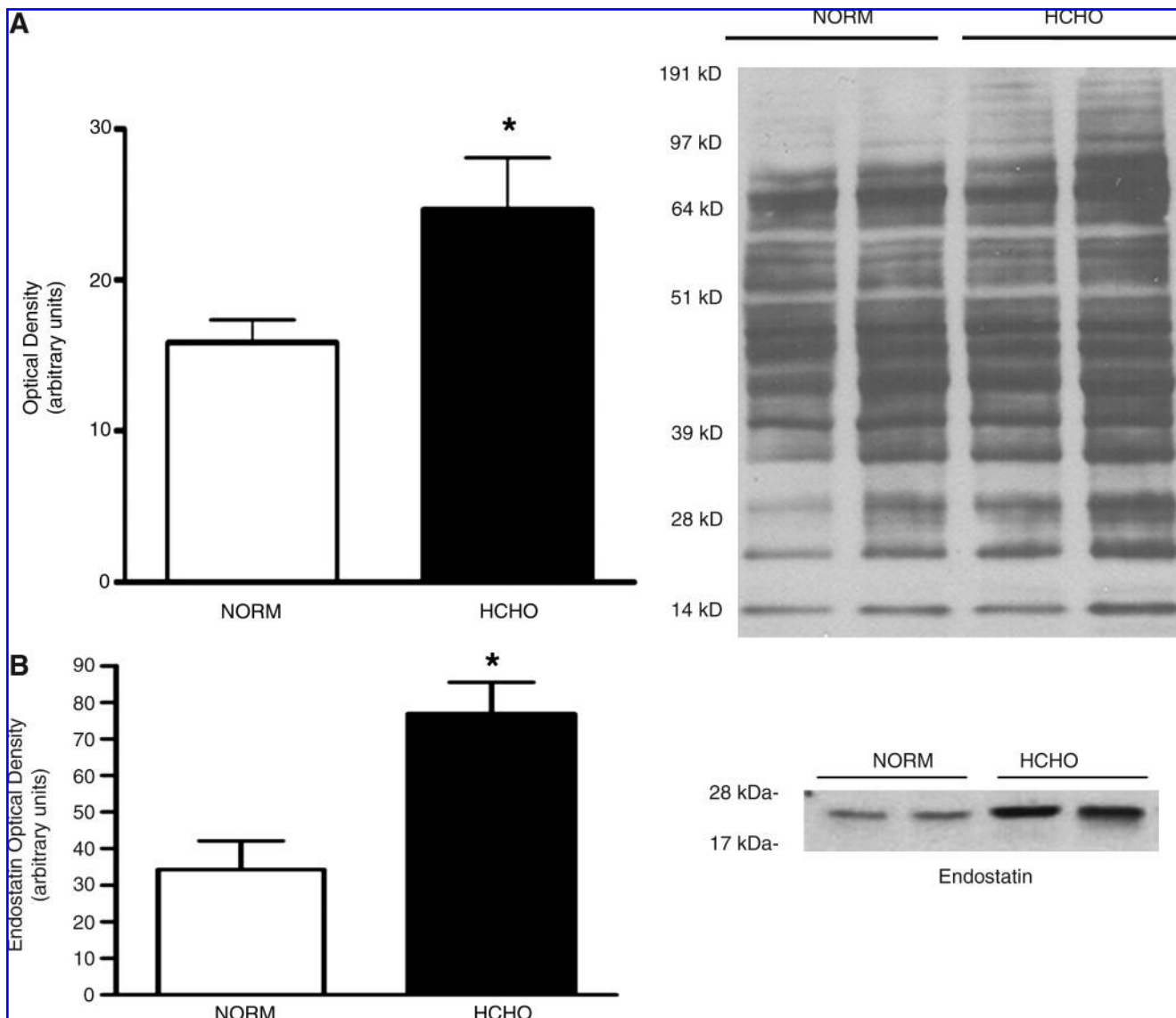


FIG. 3. Molecular findings in the setting of hypercholesterolemia. These included (A) increased protein oxidation, and (B) increased expression of endostatin in hypercholesterolemic (HCHO) versus normocholesterolemic (NORM) animals.

endostatin is antiatherosclerosis. In 1999, Moulton *et al.* (71) investigated endostatin as well as TNP-470, another substance known to inhibit the growth of capillaries, in apolipoprotein E-deficient mice fed a high-cholesterol diet for 16 weeks. They found that endostatin significantly reduced intimal neovascularization and plaque growth. More recently, Moulton *et al.* (72) indicated that loss of collagen XVIII, the source of endostatin, enhanced neovascularization of aorta in the collagen XVIII-knockout mouse. Hence, it is possible that endostatin is an important endogenous protective factor against atherosclerosis in hypercholesterolemia. Nevertheless, endostatin is very likely responsible, at least in part, for the blunted endogenous and exogenous angiogenic response in the setting of hypercholesterolemia.

Last, when the relative efficacies of therapeutic doses of VEGF and FGF-2 were compared in a hypercholesterolemic model, FGF-2 resulted in greater perfusion of the collateral-dependent territory compared with VEGF (17). Interestingly,

when evaluated in healthy, normocholesterolemic swine, the effects of intramyocardially delivered VEGF and FGF-2 are not significantly different (47). However, in a model of hypercholesterolemic endothelial dysfunction, FGF-2 appears to be a more effective agent for therapeutic angiogenesis. Possible explanation for this finding may be that VEGF-induced angiogenesis may be more NO dependent and that reduced NO bioavailability, due to increased ROS in this model, makes VEGF less effective. In addition, endostatin has significant inhibitory effects that are specific to VEGF signaling, and its increased expression in this model may be responsible for the reduced angiogenic effect of VEGF.

Diabetes and myocardial angiogenesis

More than 35 million people in the United States are affected by diabetes and glucose intolerance. These individuals carry up to 8 times the risk of cardiovascular events compared

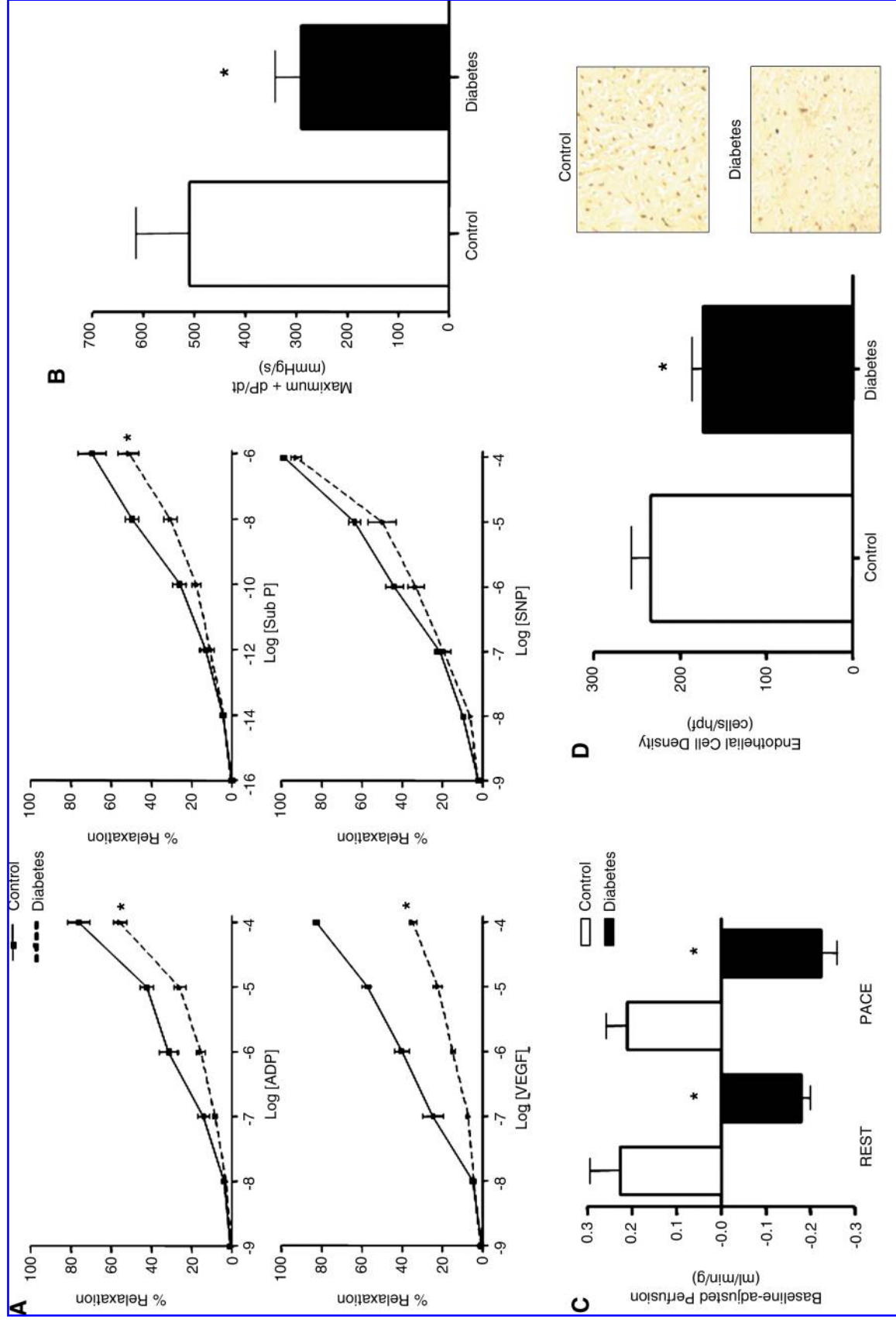


FIG. 4. Summary of functional changes in a swine model of alloxan-induced diabetes and chronic myocardial ischemia. Diabetic animals (DM) exhibited (A) endothelial dysfunction, (B) impaired left ventricular systolic function, (C) reduced collateral-dependent perfusion during rest and pacing, and (D) reduced microvessel density in the ischemic territory compared with nondiabetic controls. ADP, adenosine diphosphate; Sub P, substance P; VEGF, vascular endothelial growth factor; SNP, sodium nitroprusside. * $p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.elsevier.com/locate/yjcard).

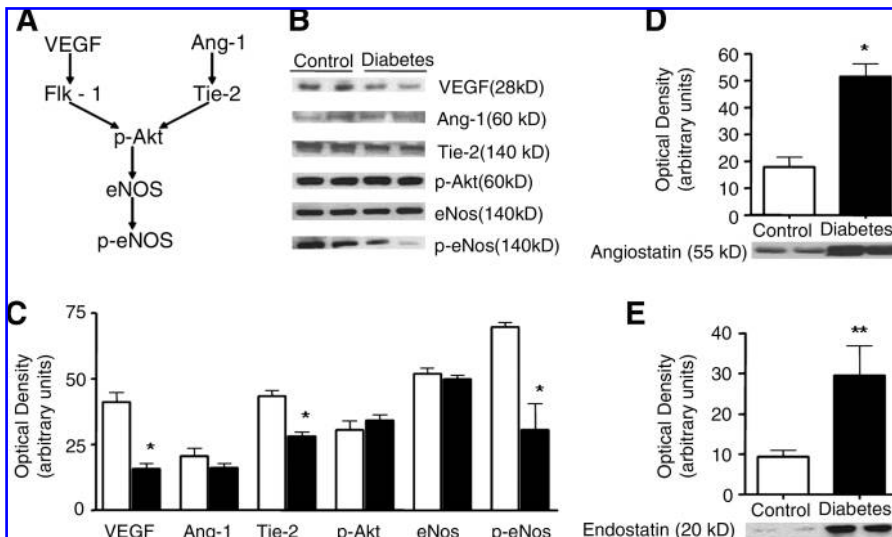


FIG. 5. (A) Schematic of proangiogenic signaling pathway, and (B) expression, and (C) quantification of proangiogenic mediators. Diabetic swine demonstrated reduced VEGF, Tie-2, and phospho-eNOS expression. Expression of antiangiogenic proteins, angiostatin (D), and endostatin (E) was significantly elevated in diabetic swine. VEGF, vascular endothelial growth factor; Ang-1, angiopoietin-1; eNOS, endothelial nitric oxide synthase.

with individuals without diabetes, making cardiovascular disease the largest cause of mortality in this population (39). Diabetic patients have accelerated atherosclerosis and also exhibit a diminished angiogenic response to myocardial ischemia, as shown angiographically (3) and in autopsy studies (128). Numerous abnormalities are found at the functional, microvascular, and molecular levels that contribute to this impaired angiogenic response.

Endothelial dysfunction in the presence of diabetes has been demonstrated in various murine (79, 103), rodent (84, 85, 104) and swine models (7) of streptozotocin (STZ)-induced diabetes as well as in coronary and noncoronary vasculature of humans with type I (20, 49, 73) and type II diabetes (68, 80, 91, 116, 121, 126). Although various mechanisms for endothelial dysfunction in diabetes have been proposed, the most commonly invoked mechanism is that of increased oxidative stress. Despite the mildly increased NOS expression and activity demonstrated in *in vitro* and *ex vivo* studies of diabetic animals, inactivation of NO by rapid reaction with reactive oxygen species appears to account for decreased NO bioavailability and impaired endothelium-dependent relaxation (19, 52, 84, 85, 102). Rosen *et al.* (84, 85) demonstrated that this increased oxidative stress in diabetic rat hearts coincides with increased levels of urine nitrites. Endothelial dysfunction seen in these studies was reversed by the administration of antioxidants like superoxide dismutase and tocopherol. Consistent with this hypothesis, increased superoxide production and increased NADPH activity has been demonstrated in coronary arteries of diabetic swine (129). Furthermore, in a diabetic rat model of hindlimb ischemia, Hirata *et al.* (46) directly correlated a diminished angiogenic response to bone marrow cell implantation to reduced plasma levels of bioavailable NO. The formation of reactive oxygen and nitrogen species, like peroxynitrite, with subsequent increase in nitrotyrosine activates poly(ADP-ribose) polymerase (PARP), a DNA-repair enzyme, which initiates an energy-consuming cycle resulting in cellular dysfunction (79, 103). Activation of PARP also induces transcription factor NF- κ B, leading to the activation of a number of pro-inflammatory cytokines such as ICAM-1 and TNF- α , which have also been implicated in diabetic endothelial dysfunction. In addition to the decreased NO bioavailability due to increased oxidative stress, other pro-

posed mechanisms for diabetic endothelial dysfunction include the uncoupling of the homodimeric eNOS (116), leading to reduced NO production, a reduced synthesis of vasodilatory prostacyclin (97), and endothelial cell dysfunction due to the chronic inflammatory state, characterized by elevated levels of inflammatory cytokines.

The dynamic involvement of the extracellular matrix (ECM) in the angiogenic process was previously described (111). Myocardial ischemia, which is a potent stimulus for collateral vessel formation, is associated with an increased breakdown of the various components of the ECM through an increased expression of elastase and matrix metalloproteinases (MMPs) and downregulation of tissue inhibitors of metalloproteinases (TIMPs). *In vitro* studies have demonstrated a diminished vascular tube formation in response to growth factors in a glycated collagen matrix (56). Nonenzymatic glycation of the ECM proteins also has been shown to reduce the angiogenic response *in vivo*. Tamarat *et al.* (105) demonstrated reduced endogenous angiogenesis in a model of hindlimb ischemia in STZ-induced diabetic mice, which was reversed by the administration of aminoguanidine, an inhibitor of advanced glycation end product (AGE) formation. Weirauch *et al.* (123) studied collateral vessel formation in permanently instrumented dogs under hyperglycemic conditions and demonstrated reduced collateral-dependent perfusion associated with increased MMP-9 activity and increased expression of the antiangiogenic protein, angiostatin.

Altered expression of angiogenic growth factors and related mediators also is known to occur with diabetes. Diminished activation of HIF-1 α , a transcription factor that triggers the angiogenic response, in an acute ischemia model (62, 78, 106), as well as alterations in the expression of angiopoietins and the tie-2 receptor, have been shown in diabetic rats (78). Sasso *et al.* (88) studied the expression of VEGF and its downstream mediators in myocardial biopsies of patients with or without type II diabetes and found that whereas VEGF expression was increased, VEGF-receptor activation and downstream signaling were reduced.

The effects of diabetes on the angiogenic response was studied in a large-animal model of a 15-week period of alloxan-induced diabetes in Yucatan miniswine with subsequent creation of chronic myocardial ischemia by using a circum-

flex coronary artery ameroid constrictor (16). In this model, diabetic animals successfully achieved blood glucose levels between 250 and 400 mg/dl and demonstrated significant impairments in coronary microvascular relaxation to ADP and substance P. Furthermore, microvascular relaxation in response to VEGF was even further reduced, suggesting an impairment in VEGF signaling beyond the reduction in bioavailable NO. Collateral-dependent perfusion in the circumflex territory was profoundly reduced and was also associated with reduced left ventricular function. Consistent with the finding of reduced perfusion and function, diabetic animals had reduced vascular density compared with normoglycemic controls in the ischemic territory (Fig. 4). Together, these findings demonstrate an impaired endogenous angiogenic response to chronic myocardial ischemia.

At the molecular level, the myocardium of these animals exhibited reduced expression of VEGF and of the angiopoietin receptor, Tie-2, as well as reduced phosphorylation and activation of eNOS. Expression of antiangiogenic proteins, endostatin (about a threefold increase) and particularly, angiostatin (about a 4.5-fold increase) was also significantly elevated in diabetic animals *versus* controls (Fig. 5). Overall, this large-animal model successfully characterized the functional, microvascular, and molecular abnormalities that have been observed in human diabetes and offers an opportunity to evaluate novel therapeutic strategies to enhance the angiogenic response in the setting of diabetes.

Role of Substrate Modification

In response to limited therapeutic efficacy of growth factor-based angiogenic therapy in clinical trials, researchers have investigated a number of strategies to enhance the angiogenic response. These include the search for a more-potent angiogenic agent or combination of agents, improved and sustained delivery strategies that may involve sustained-release devices or gene-based or cell-based delivery, as well as more-targeted delivery strategies (*i.e.*, intramyocardial and perivascular delivery compared with intravenous or intracoronary routes). In addition, some investigators explored substances that can modify the substrate to improve the response to the therapeutic angiogenic agent. Substrate-modifying strategies are briefly discussed below.

L-Arginine

Numerous studies have clearly demonstrated that the presence of endothelial dysfunction is associated with impaired endogenous and exogenous angiogenic responses. This endothelial dysfunction is secondary to a reduction in bioavailable NO, which may occur because of reduced NO production or increased consumption. L-Arginine, the substrate for endothelial nitric oxide synthase, can increase the amount of bioavailable NO by increasing its production. Evidence for its pro-endothelial effects comes from animal studies as well as demonstration of improved coronary vasorelaxation in response to acetylcholine in patients with coronary disease (83). In the context of angiogenesis, studies by Jang (48) and Duan (30) in a hypercholesterolemic mouse model of hindlimb ischemia were the first to demonstrate amelioration of a blunted angiogenic response to FGF-2 administration *in vivo* through dietary supplementation with L-arginine. These concepts have since been validated in a large-animal model of hypercholes-

terolemic swine. In these animals, oral supplementation with L-arginine reversed endothelial dysfunction and improved collateral-dependent perfusion in response to chronic myocardial ischemia (75), as well as with VEGF (117) and FGF-2 (119) supplementation. These findings have been further translated to the clinical setting in a trial in which patients undergoing coronary artery bypass surgery with at least one non-revascularizable territory were randomly assigned to receive placebo, VEGF alone, L-arginine alone, or VEGF and L-arginine in a factorial trial design (86). Although small in size, this study demonstrated trends toward smaller perfusion defects and trends toward improved angina scores in the combination-therapy group. Contrary to these findings, the VINTAGE MI clinical trial (90), which randomized patients to L-arginine *versus* placebo after acute myocardial infarction, had increased mortality in the L-arginine group. It is important to recognize in this context that patients with acute myocardial infarction are a very different patient population compared with patients with chronic stable coronary disease and that L-arginine supplementation may still hold promise as an adjunctive therapy for therapeutic angiogenesis. Finally, it should be noted that L-arginine is also a substrate for arginase and leads to the formation of polyamines, and the role of this pathway in the setting of angiogenesis has not been well elucidated.

Statins

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are commonly used in patients with coronary disease. In addition to inhibiting the rate-limiting step in cholesterol biosynthesis, statins appear to have a number of off-target or pleiotropic effects. Statins have been shown to improve peripheral and coronary endothelial dysfunction in coronary artery disease patients (8). They can increase NO bioavailability by activating Akt, which subsequently leads to endothelial nitric oxide synthase (eNOS) activation (94), as well as through its antioxidant effects (63). Interestingly, *in vitro* and murine studies have suggested a biphasic and dose-dependent effect of statins on angiogenesis (114, 124). In a swine model of hypercholesterolemia, oral supplementation with high-dose atorvastatin reversed the hypercholesterolemia-induced endothelial dysfunction, as demonstrated by normalization of microvessel relaxation to ADP and VEGF. However, improvements in endothelial function did not lead to improved endogenous angiogenic response to chronic myocardial ischemia (14) or to exogenous VEGF protein administration (12). In this model, atorvastatin-treated animals had significantly and persistently elevated levels of Akt phosphorylation, a known effect of statins, as well as increased expression of the antiangiogenic protein, endostatin. These abnormalities were also replicated in normocholesterolemic swine (11). These studies communicate that, in addition to endothelial function, other determinants of the angiogenic response can affect the response to substrate-modifying therapies.

Insulin

Glycemic control remains the mainstay of treatment in diabetes and has been shown to improve both macrovascular and microvascular complications of diabetes (1, 2). Insulin treatment, with or without oral hypoglycemic agents, is the

most common method used clinically to achieve glycemic control. Insulin has multifaceted effects on the myocardium, which mainly involve the regulation of fuel consumption, glucose transport, glycogen synthesis, and glycolysis (18). More relevant to vascular function, however, is the demonstrated ability of insulin to increase endothelial nitric oxide (NO) availability in the vasculature (125). Furthermore, the insulin receptor, which is present in the myocardium, is a tyrosine kinase receptor that shares many of the downstream mediators common to angiogenic growth factors and their receptors [e.g., PI3 kinase and MAP kinases (125)]. In addition to its direct effects on the myocardium and coronary vasculature, insulin exerts indirect effects through the reduction in systemic blood glucose levels. By reducing blood glucose levels, insulin can avoid the adverse effects of chronic hyperglycemia, which include increased oxidative stress, chronic inflammation, and the nonenzymatic glycation of proteins, particularly in the extracellular matrix (122, 129). In the large-animal model of alloxan-induced diabetes and chronic circumflex territory myocardial ischemia described earlier, parenteral insulin treatment was successfully used to achieve glycemic control (fasting blood glucose, <150 mg/dl) (15). Insulin treatment resulted in significant improvements in, but not normalization of, diabetic endothelial dysfunction. Significant improvements also were observed in collateral-dependent perfusion, as well as systolic and diastolic left ventricular function and capillary density in the ischemic territory. These functional findings of an improved endogenous angiogenic response were accompanied by increased expression of proangiogenic growth factors VEGF and angiopoietin-1, as well as its receptor, Tie-2. Last, the expression of the antiangiogenic proteins, angiostatin and endostatin, which were significantly elevated in the setting of diabetes, was reduced with insulin therapy. Thus, insulin treatment holds significant potential as a substrate-modulating agent for therapeutic angiogenesis in the setting of diabetes.

Other agents

A number of agents hold some potential as substrate-modifying agents for therapeutic angiogenesis because of either their pro-endothelial or antioxidant properties. Ascorbic acid and α -tocopherol (vitamins C and E) have demonstrated antioxidant properties that may be useful in improving endothelial function in certain disease states. Preliminary evidence from rodent models of hindlimb ischemia suggests that supplementation with vitamins C and E, along with L-arginine, may enhance the angiogenic effect of bone marrow cell infusion (76). Resveratrol, an ingredient found in red wine, has been shown to have antioxidant and cardioprotective effects in a variety of disease states and may have a beneficial effect on angiogenesis (26, 107). Other agents that have pro-endothelial properties and may play a role in enhancing therapeutic angiogenesis include angiotensin-converting enzyme (ACE) inhibitors, PPAR- γ agonists, and other oral hypoglycemic agents. These substances must be studied in large-animal models, to better elucidate their effects on the coronary vasculature and the angiogenic response.

Conclusions

For more than a decade, cardiovascular researchers and clinicians have explored therapeutic angiogenesis by using

growth factors or cell-based therapies as treatment options for patients with advanced coronary artery disease. A number of therapeutic agents have undergone extensive preclinical evaluation followed by phase I, II, and III clinical trials. Despite encouraging results in animal models, clinical trials have showed minimal measurable benefits in patients. These investigations, however, have given us a new level of insight into the complexity of the angiogenic process and determinants of therapeutic success. For angiogenic therapy to become a clinically viable therapeutic option, it will have to involve modulation of multiple potent growth factors and be delivered in a targeted manner to the desired territory, with a sustained effect and without major adverse effects. Additionally, the therapeutic strategy will have to address the antiangiogenic influences present in the host tissue. Improved understanding of factors influencing the substrate with approaches for substrate modification will likely be an important part of this therapeutic strategy.

Acknowledgments

We are grateful for financial support from grant R01 HL69024 from the National Institutes of Health (Dr. Sellke).

Abbreviations

ACE, Angiotensin-converting enzyme; ADP, adenosine diphosphate; AGE, advanced glycation end product; CAD, coronary artery disease; CABG, coronary artery bypass graft; DNA, deoxyribonucleic acid; ECM, extracellular matrix; FGF, fibroblast growth factor; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ICAM, intracellular adhesion molecule; L-NAME, N^G -nitro-L-arginine methyl ester; MMP, matrix metalloproteinase; mRNA, messenger ribonucleic acid; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NO, nitric oxide; NOS, nitric oxide synthase; NNA, N^G -nitro-L-arginine; PARP, poly(ADP-ribose) polymerase; PCI, percutaneous coronary intervention; PDGF, platelet-derived growth factor; PIGF, placental growth factor; ROS, reactive oxygen species; STZ, streptozotocin; TIMP, tissue inhibitors of metalloproteinases; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.

References

1. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care* 28: S4–S36, 2005.
2. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus: the Diabetes Control and Complications Trial Research Group. *N Engl J Med* 329: 977–986, 1993.
3. Abaci A, Oguzhan A, Kahraman S, Eryol NK, Unal S, Arinc H, and Ergin A. Effect of diabetes mellitus on formation of coronary collateral vessels. *Circulation* 99: 2239–2242, 1999.
4. Arnal JF, Yamin J, Dockery S, and Harrison DG. Regulation of endothelial nitric oxide synthase mRNA, protein, and activity during cell growth. *Am J Physiol* 267: C1381–C1388, 1994.
5. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, and Isner JM. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-

- derived endothelial progenitor cells. *EMBO J* 18: 3964–3972, 1999.
6. Babaei S, Teichert-Kuliszewska K, Monge JC, Mohamed F, Bendeck MP, and Stewart DJ. Role of nitric oxide in the angiogenic response in vitro to basic fibroblast growth factor. *Circ Res* 82: 1007–1015, 1998.
 7. Bagwell CA and Brophy C. Enhanced arterial contractile responses in diabetic hypercholesterolemic pig carotid arteries. *Int J Surg Invest* 1: 477–481, 2000.
 8. Balk EM, Karas RH, Jordan HS, Kupelnick B, Chew P, and Lau J. Effects of statins on vascular structure and function: a systematic review. *Am J Med* 117: 775–790, 2004.
 9. Bernotat-Danielowski S, Sharma HS, Schott RJ, and Schaper W. Generation and localisation of monoclonal antibodies against fibroblast growth factors in ischaemic collateralised porcine myocardium. *Cardiovasc Res* 27: 1220–1228, 1993.
 10. Bonow RO, Smaha LA, Smith SC, Jr., Mensah GA, and Lenfant C. World Heart Day 2002: the international burden of cardiovascular disease: responding to the emerging global epidemic. *Circulation* 106: 1602–1605, 2002.
 11. Boodhwani M, Mieno S, Feng J, Sodha NR, Clements RT, Xu SH, and Sellke FW. Atorvastatin impairs the myocardial angiogenic response to chronic ischemia in normocholesterolemic swine. *J Thorac Cardiovasc Surg* 135: 117–122, 2008.
 12. Boodhwani M, Mieno S, Voisine P, Feng J, Sodha N, Li J, and Sellke FW. High-dose atorvastatin is associated with impaired myocardial angiogenesis in response to vascular endothelial growth factor in hypercholesterolemic swine. *J Thorac Cardiovasc Surg* 132: 1299–1306, 2006.
 13. Boodhwani M, Nakai Y, Mieno S, Voisine P, Bianchi C, Araujo EG, Feng J, Michael K, Li J, and Sellke FW. Hypercholesterolemia impairs the myocardial angiogenic response in a swine model of chronic ischemia: role of endostatin and oxidative stress. *Ann Thorac Surg* 81: 634–641, 2006.
 14. Boodhwani M, Nakai Y, Voisine P, Feng J, Li J, Mieno S, Ramlawi B, Bianchi C, Laham R, and Sellke FW. High-dose atorvastatin improves hypercholesterolemic coronary endothelial dysfunction without improving the angiogenic response. *Circulation* 114: I402–I408, 2006.
 15. Boodhwani M, Sodha NR, Mieno S, Ramlawi B, Xu SH, Feng J, Clements RT, Ruel M, and Sellke FW. Insulin treatment enhances the myocardial angiogenic response in diabetes. *J Thorac Cardiovasc Surg* 134: 1453–1460, 2007. Epub November 5, 2007.
 16. Boodhwani M, Sodha NR, Mieno S, Xu SH, Feng J, Ramlawi B, Clements RT, and Sellke FW. Functional, cellular, and molecular characterization of the angiogenic response to chronic myocardial ischemia in diabetes. *Circulation* 116 (Suppl 11): 131–137, 2007.
 17. Boodhwani M, Voisine P, Ruel M, Sodha NR, Feng J, Xu SH, Bianchi C, and Sellke FW. Comparison of vascular endothelial growth factor and fibroblast growth factor-2 in a swine model of endothelial dysfunction. *Eur J Cardiothorac Surg* 33: 645–650, discussion 251–252, 2008.
 18. Brownsey RW, Boone AN, and Allard MF. Actions of insulin on the mammalian heart: metabolism, pathology and biochemical mechanisms. *Cardiovasc Res* 34: 3–24, 1997.
 19. Cai H and Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 87: 840–844, 2000.
 20. Calver A, Collier J, and Vallance P. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J Clin Invest* 90: 2548–2554, 1992.
 21. Carmeliet P. Angiogenesis in health and disease. *Nat Med* 9: 653–660, 2003.
 22. Carmeliet P. Manipulating angiogenesis in medicine. *J Intern Med* 255: 538–561, 2004.
 23. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 6: 389–395, 2000.
 24. Casscells W, Speir E, Sasse J, Klagsbrun M, Allen P, Lee M, Calvo B, Chiba M, Haggroth L, Folkman J, and *et al.* Isolation, characterization, and localization of heparin-binding growth factors in the heart. *J Clin Invest* 85: 433–441, 1990.
 25. Cohen RA, Zitnay KM, Haudenschild CC, and Cunningham LD. Loss of selective endothelial cell vasoactive functions caused by hypercholesterolemia in pig coronary arteries. *Circ Res* 63: 903–910, 1988.
 26. Csiszar A, Labinskyy N, Podlitsky A, Kaminski PM, Wolin MS, Zhang C, Mukhopadhyay P, Pacher P, Hu F, de Cabo R, Ballabh P, and Ungvari Z. Vasoprotective effects of resveratrol and SIRT1: attenuation of cigarette smoke-induced oxidative stress and proinflammatory phenotypic alterations. *Am J Physiol Heart Circ Physiol* 294: H2721–H2735, 2008.
 27. Cuevas P, Carceller F, Ortega S, Zazo M, Nieto I, and Gimenez-Gallego G. Hypotensive activity of fibroblast growth factor. *Science* 254: 1208–1210, 1991.
 28. de Wilt JH, Manusama ER, van Etten B, van Tiel ST, Jorna AS, Seynhaeve AL, ten Hagen TL, and Eggermont AM. Nitric oxide synthase inhibition results in synergistic antitumour activity with melphalan and tumour necrosis factor alpha-based isolated limb perfusions. *Br J Cancer* 83: 1176–1182, 2000.
 29. Detillieux KA, Sheikh F, Kardami E, and Cattini PA. Biological activities of fibroblast growth factor-2 in the adult myocardium. *Cardiovasc Res* 57: 8–19, 2003.
 30. Duan J, Murohara T, Ikeda H, Katoh A, Shintani S, Sasaki K, Kawata H, Yamamoto N, and Imaizumi T. Hypercholesterolemia inhibits angiogenesis in response to hindlimb ischemia: nitric oxide-dependent mechanism. *Circulation* 102: III370–III376, 2000.
 31. Eliceiri BP, Paul R, Schwartzberg PL, Hood JD, Leng J, and Chersesh DA. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. *Mol Cell* 4: 915–924, 1999.
 32. Ellis SG, Chew D, Chan A, Whitlow PL, Schneider JP, and Topol EJ. Death following creatine kinase-MB elevation after coronary intervention: identification of an early risk period: importance of creatine kinase-MB level, completeness of revascularization, ventricular function, and probable benefit of statin therapy. *Circulation* 106: 1205–1210, 2002.
 33. Esper RJ, Vilarino J, Cacharron JL, Machado R, Ingino CA, Garcia Guinazu CA, Bereziuk E, Bolano AL, Suarez DH, and Kura M. Impaired endothelial function in patients with rapidly stabilized unstable angina: assessment by noninvasive brachial artery ultrasonography. *Clin Cardiol* 22: 699–703, 1999.
 34. Faham S, Hileman RE, Fromm JR, Linhardt RJ, and Rees DC. Heparin structure and interactions with basic fibroblast growth factor. *Science* 271: 1116–1120, 1996.
 35. Folkman J. Angiogenic therapy of the human heart. *Circulation* 97: 628–629, 1998.
 36. Fukuchi M and Giaid A. Endothelial expression of endothelial nitric oxide synthase and endothelin-1 in human coronary artery disease: specific reference to underlying lesion. *Lab Invest* 79: 659–670, 1999.

37. Grines CL, Watkins MW, Helmer G, Penny W, Brinker J, Marmur JD, West A, Rade JJ, Marrott P, Hammond HK, and Engler RL. Angiogenic Gene Therapy (AGENT) trial in patients with stable angina pectoris. *Circulation* 105: 1291–1297, 2002.
38. Grines CL, Watkins MW, Mahmarian JJ, Iskandrian AE, Rade JJ, Marrott P, Pratt C, and Kleiman N. A randomized, double-blind, placebo-controlled trial of Ad5FGF-4 gene therapy and its effect on myocardial perfusion in patients with stable angina. *J Am Coll Cardiol* 42: 1339–1347, 2003.
39. Grundy SM, Garber A, Goldberg R, Havas S, Holman R, Lamendola C, Howard WJ, Savage P, Sowers J, and Vega GL. Prevention Conference VI: Diabetes and Cardiovascular Disease: writing group IV: lifestyle and medical management of risk factors. *Circulation* 105: e153–e158, 2002.
40. Hasdai D, Mathew V, Schwartz RS, Holmes DR Jr, and Lerman A. The effect of basic fibroblast growth factor on coronary vascular tone in experimental hypercholesterolemia in vivo and in vitro. *Coron Artery Dis* 8: 299–304, 1997.
41. Hasdai D, Mathew V, Schwartz RS, Smith LA, Holmes DR Jr, Katusic ZS, and Lerman A. Enhanced endothelin-B-receptor-mediated vasoconstriction of small porcine coronary arteries in diet-induced hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 17: 2737–2743, 1997.
42. Hasdai D, Nielsen MF, Rizza RA, Holmes DR Jr, Richardson DM, Cohen P, and Lerman A. Attenuated in vitro coronary arteriolar vasorelaxation to insulin-like growth factor I in experimental hypercholesterolemia. *Hypertension* 34: 89–95, 1999.
43. Hedman M, Hartikainen J, Syvanne M, Stjernvall J, Hedman A, Kivela A, Vanninen E, Mussalo H, Kauppila E, Simula S, Narvanen O, Rantala A, Peuhkurinen K, Nieminen MS, Laakso M, and Yla-Herttuala S. Safety and feasibility of catheter-based local intracoronary vascular endothelial growth factor gene transfer in the prevention of post-angioplasty and in-stent restenosis and in the treatment of chronic myocardial ischemia: phase II results of the Kuopio Angiogenesis Trial (KAT). *Circulation* 107: 2677–2683, 2003.
44. Henry TD and Abraham JA. Review of preclinical and clinical results with vascular endothelial growth factors for therapeutic angiogenesis. *Curr Intervent Cardiol Rep* 2: 228–241, 2000.
45. Henry TD, Annex BH, McKendall GR, Azrin MA, Lopez JJ, Giordano FJ, Shah PK, Willerson JT, Benza RL, Berman DS, Gibson CM, Bajamonde A, Rundle AC, Fine J, and McCluskey ER. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. *Circulation* 107: 1359–1365, 2003.
46. Hirata K, Li TS, Nishida M, Ito H, Matsuzaki M, Kasaoka S, and Hamano K. Autologous bone marrow cell implantation as therapeutic angiogenesis for ischemic hindlimb in diabetic rat model. *Am J Physiol Heart Circ Physiol* 284: H66–H70, 2003.
47. Hughes GC, Biswas SS, Yin B, Coleman RE, DeGrado TR, Landolfo CK, Lowe JE, Annex BH, and Landolfo KP. Therapeutic angiogenesis in chronically ischemic porcine myocardium: comparative effects of bFGF and VEGF. *Ann Thorac Surg* 77: 812–818, 2004.
48. Jang JJ, Ho HK, Kwan HH, Fajardo LF, and Cooke JP. Angiogenesis is impaired by hypercholesterolemia: role of asymmetric dimethylarginine. *Circulation* 102: 1414–1419, 2000.
49. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, and Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 88: 2510–2516, 1993.
50. Jones EL, Craver JM, Guyton RA, Bone DK, Hatcher CR Jr, and Riechwald N. Importance of complete revascularization in performance of the coronary bypass operation. *Am J Cardiol* 51: 7–12, 1983.
51. Jozkowicz A, Cooke JP, Guevara I, Huk I, Funovics P, Pachinger O, Weidinger F, and Dulak J. Genetic augmentation of nitric oxide synthase increases the vascular generation of VEGF. *Cardiovasc Res* 51: 773–783, 2001.
52. Karasu C. Time course of changes in endothelium-dependent and -independent relaxation of chronically diabetic aorta: role of reactive oxygen species. *Eur J Pharmacol* 392: 163–173, 2000.
53. Kastrup J, Jorgensen E, Ruck A, Tagil K, Glogar D, Ruzyllo W, Botker HE, Dudek D, Drvota V, Hesse B, Thuesen L, Blomberg P, Gyongyosi M, and Sylven C. Direct intramyocardial plasmid vascular endothelial growth factor-A165 gene therapy in patients with stable severe angina pectoris: a randomized double-blind placebo-controlled study: the Euroinject One trial. *J Am Coll Cardiol* 45: 982–988, 2005.
54. Kaufmann PA, Gnecci-Ruscone T, Schafers KP, Luscher TF, and Camici PG. Low density lipoprotein cholesterol and coronary microvascular dysfunction in hypercholesterolemia. *J Am Coll Cardiol* 36: 103–109, 2000.
55. Kleisli T, Cheng W, Jacobs MJ, Mirocha J, Derobertis MA, Kass RM, Blanche C, Fontana GP, Raissi SS, Magliato KE, and Trento A. In the current era, complete revascularization improves survival after coronary artery bypass surgery. *J Thorac Cardiovasc Surg* 129: 1283–1291, 2005.
56. Kuzuya M, Satake S, Ai S, Asai T, Kanda S, Ramos MA, Miura H, Ueda M, and Iguchi A. Inhibition of angiogenesis on glycated collagen lattices. *Diabetologia* 41: 491–499, 1998.
57. Laham R and Simons M. Growth factor therapy in ischemic heart disease. In: Rubanyi G, editor. *Angiogenesis in health and disease*. New York: Marcel Dekker; 2000, pp 451–475.
58. Laham RJ, Sellke FW, Edelman ER, Pearlman JD, Ware JA, Brown DL, Gold JP, and Simons M. Local perivascular delivery of basic fibroblast growth factor in patients undergoing coronary bypass surgery: results of a phase I randomized, double-blind, placebo-controlled trial. *Circulation* 100: 1865–1871, 1999.
59. Li JM and Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. *Am J Physiol Regul Integr Comp Physiol* 287: R1014–R1030, 2004.
60. Losordo DW, Vale PR, Hendel RC, Milliken CE, Fortuin FD, Cummings N, Schatz RA, Asahara T, Isner JM, and Kuntz RE. Phase 1/2 placebo-controlled, double-blind, dose-escalating trial of myocardial vascular endothelial growth factor 2 gene transfer by catheter delivery in patients with chronic myocardial ischemia. *Circulation* 105: 2012–2018, 2002.
61. Ma L and Wallace JL. Endothelial nitric oxide synthase modulates gastric ulcer healing in rats. *Am J Physiol Gastrointest Liver Physiol* 279: G341–G346, 2000.
62. Marfella R, D'Amico M, Di Filippo C, Piegari E, Nappo F, Esposito K, Berrino L, Rossi F, and Giugliano D. Myocardial infarction in diabetic rats: role of hyperglycaemia on infarct size and early expression of hypoxia-inducible factor 1. *Diabetologia* 45: 1172–1181, 2002.

63. Mason RP, Walter MF, and Jacob RF. Effects of HMG-CoA reductase inhibitors on endothelial function: role of microdomains and oxidative stress. *Circulation* 109: II34–II41, 2004.
64. Maulik N. Reactive oxygen species drives myocardial angiogenesis? *Antioxid Redox Signal* 8: 2161–2168, 2006.
65. Maulik N. Redox signaling of angiogenesis. *Antioxid Redox Signal* 4: 805–815, 2002.
66. Maulik N and Das DK. Redox signaling in vascular angiogenesis. *Free Radic Biol Med* 33: 1047–1060, 2002.
67. McNeer JF, Conley MJ, Starmer CF, Behar VS, Kong Y, Peter RH, Bartel AG, Oldham HN Jr, Young WG Jr, Sabiston DC Jr, and Rosati RA. Complete and incomplete revascularization at aortocoronary bypass surgery: experience with 392 consecutive patients. *Am Heart J* 88: 176–182, 1974.
68. Momose M, Abletshauser C, Neverve J, Nekolla SG, Schnell O, Standl E, Schwaiger M, and Bengel FM. Dysregulation of coronary microvascular reactivity in asymptomatic patients with type 2 diabetes mellitus. *Eur J Nucl Med Mol Imaging* 29: 1675–1679, 2002.
69. Moon MR, Sundt TM 3rd, Pasque MK, Barner HB, Gay WA Jr, and Damiano RJ Jr. Influence of internal mammary artery grafting and completeness of revascularization on long-term outcome in octogenarians. *Ann Thorac Surg* 72: 2003–2007, 2001.
70. Morbidelli L, Chang CH, Douglas JG, Granger HJ, Ledda F, and Ziche M. Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. *Am J Physiol* 270: H411–H415, 1996.
71. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, and Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. *Circulation* 99: 1726–1732, 1999.
72. Moulton KS, Olsen BR, Sonn S, Fukai N, Zurakowski D, and Zeng X. Loss of collagen XVIII enhances neovascularization and vascular permeability in atherosclerosis. *Circulation* 110: 1330–1336, 2004.
73. Mullen MJ, Clarkson P, Donald AE, Thomson H, Thorne SA, Powe AJ, Furuno T, Bull T, and Deanfield JE. Effect of enalapril on endothelial function in young insulin-dependent diabetic patients: a randomized, double-blind study. *J Am Coll Cardiol* 31: 1330–1335, 1998.
74. Murohara T, Witzenbichler B, Spyridopoulos I, Asahara T, Ding B, Sullivan A, Losordo DW, and Isner JM. Role of endothelial nitric oxide synthase in endothelial cell migration. *Arterioscler Thromb Vasc Biol* 19: 1156–1161, 1999.
75. Nakai Y, Voisine P, Bianchi C, Xu SH, Feng J, Malik T, Rosinberg A, and Sellke FW. Effects of L-arginine on the endogenous angiogenic response in a model of hypercholesterolemia. *Surgery* 138: 291–298, 2005.
76. Napoli C, Williams-Ignarro S, de Nigris F, de Rosa G, Lerman LO, Farzati B, Matarazzo A, Sica G, Botti C, Fiore A, Byrns RE, Sumi D, Sica V, and Ignarro LJ. Beneficial effects of concurrent autologous bone marrow cell therapy and metabolic intervention in ischemia-induced angiogenesis in the mouse hindlimb. *Proc Natl Acad Sci U S A* 102: 17202–17206, 2005.
77. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, and Folkman J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88: 277–285, 1997.
78. Ohashi H, Takagi H, Koyama S, Oh H, Watanabe D, Antonetti DA, Matsubara T, Nagai K, Arai H, Kita T, and Honda Y. Alterations in expression of angiopoietins and the Tie-2 receptor in the retina of streptozotocin induced diabetic rats. *Mol Vis* 10: 608–617, 2004.
79. Pacher P, Liaudet L, Soriano FG, Mabley JG, Szabo E, and Szabo C. The role of poly(ADP-ribose) polymerase activation in the development of myocardial and endothelial dysfunction in diabetes. *Diabetes* 51: 514–521, 2002.
80. Papaioannou GI, Seip RL, Grey NJ, Katten D, Taylor A, Inzucchi SE, Young LH, Chyun DA, Davey JA, Wackers FJ, Iskandrian AE, Ratner RE, Robinson EC, Carolan S, Engel S, and Heller GV. Brachial artery reactivity in asymptomatic patients with type 2 diabetes mellitus and microalbuminuria (from the Detection of Ischemia in Asymptomatic Diabetics: Brachial Artery Reactivity Study). *Am J Cardiol* 94: 294–299, 2004.
81. Papapetropoulos A, Desai KM, Rudic RD, Mayer B, Zhang R, Ruiz-Torres MP, Garcia-Cardena G, Madri JA, and Sessa WC. Nitric oxide synthase inhibitors attenuate transforming-growth-factor-beta 1-stimulated capillary organization in vitro. *Am J Pathol* 150: 1835–1844, 1997.
82. Pecher P and Schumacher BA. Angiogenesis in ischemic human myocardium: clinical results after 3 years. *Ann Thorac Surg* 69: 1414–1419, 2000.
83. Quyyumi AA, Dakak N, Diodati JG, Gilligan DM, Panza JA, and Cannon RO 3rd. Effect of L-arginine on human coronary endothelium-dependent and physiologic vasodilation. *J Am Coll Cardiol* 30: 1220–1227, 1997.
84. Rosen P, Ballhausen T, Bloch W, and Addicks K. Endothelial relaxation is disturbed by oxidative stress in the diabetic rat heart: influence of tocopherol as antioxidant. *Diabetologia* 38: 1157–1168, 1995.
85. Rosen P, Ballhausen T, and Stockklauser K. Impairment of endothelium dependent relaxation in the diabetic rat heart: mechanisms and implications. *Diabetes Res Clin Pract* 31(suppl): S143–S155, 1996.
86. Ruel M, Beanlands RS, Lortie M, Chan V, Camack N, deKemp RA, Suuronen EJ, Rubens FD, DaSilva JN, Sellke FW, Stewart DJ, and Mesana TG. Concomitant treatment with oral L-arginine improves the efficacy of surgical angiogenesis in patients with severe diffuse coronary artery disease: the Endothelial Modulation in Angiogenic Therapy randomized controlled trial. *J Thorac Cardiovasc Surg* 135: 762–770, 2008.
87. Ruel M, Wu GF, Khan TA, Voisine P, Bianchi C, Li J, Laham RJ, and Sellke FW. Inhibition of the cardiac angiogenic response to surgical FGF-2 therapy in a swine endothelial dysfunction model. *Circulation* 108(suppl 1): II335–II340, 2003.
88. Sasso FC, Torella D, Carbonara O, Ellison GM, Torella M, Scardone M, Marra C, Nasti R, Marfella R, Cozzolino D, Indolfi C, Cotrufo M, Torella R, and Salvatore T. Increased vascular endothelial growth factor expression but impaired vascular endothelial growth factor receptor signaling in the myocardium of type 2 diabetic patients with chronic coronary heart disease. *J Am Coll Cardiol* 46: 827–834, 2005.
89. Schneider H and Huse K. Arterial gene therapy. *Lancet* 348: 1380–1381; author reply 1381–1382, 1996.
90. Schulman SP, Becker LC, Kass DA, Champion HC, Terrin ML, Forman S, Ernst KV, Kelemen MD, Townsend SN, Capriotti A, Hare JM, and Gerstenblith G. L-arginine therapy in acute myocardial infarction: the Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *JAMA* 295: 58–64, 2006.

91. Sekiya M, Suzuki J, Watanabe K, Funada J, Otani T, and Akutsu H. Beneficial effect of troglitazone, an insulin-sensitizing antidiabetic agent, on coronary circulation in patients with non-insulin-dependent diabetes mellitus. *Jpn Circ J* 65: 487–490, 2001.
92. Sellke FW, Laham RJ, Edelman ER, Pearlman JD, and Simons M. Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. *Ann Thorac Surg* 65: 1540–1544, 1998.
93. Sellke FW, Wang SY, Stamler A, Lopez JJ, Li J, and Simons M. Enhanced microvascular relaxations to VEGF and bFGF in chronically ischemic porcine myocardium. *Am J Physiol* 271: H713–H7120, 1996.
94. Shiojima I and Walsh K. Role of Akt signaling in vascular homeostasis and angiogenesis. *Circ Res* 90: 1243–1250, 2002.
95. Shishido T, Tasaki K, Takeishi Y, Takasaki S, Miyamoto T, Itoh M, Takahashi H, Kubota I, Ito T, Katano Y, Wakabayashi I, and Tomoike H. Chronic hypertriglyceridemia in young watanabe heritable hyperlipidemic rabbits impairs endothelial and medial smooth muscle function. *Life Sci* 74: 1487–501, 2004.
96. Sieber CC, Sumanovski LT, Stumm M, van der Kooij M, and Battegay E. In vivo angiogenesis in normal and portal hypertensive rats: role of basic fibroblast growth factor and nitric oxide. *J Hepatol* 34: 644–650, 2001.
97. Silberbauer K, Clopath P, Sinzinger H, and Schernthaner G. Effect of experimentally induced diabetes on swine vascular prostacyclin (PGI₂) synthesis. *Artery* 8: 30–36, 1980.
98. Simons M, Annex BH, Laham RJ, Kleiman N, Henry T, Dauerman H, Udelson JE, Gervino EV, Pike M, Whitehouse MJ, Moon T, and Chronos NA. Pharmacological treatment of coronary artery disease with recombinant fibroblast growth factor-2: double-blind, randomized, controlled clinical trial. *Circulation* 105: 788–793, 2002.
99. Simons M, Bonow RO, Chronos NA, Cohen DJ, Giordano FJ, Hammond HK, Laham RJ, Li W, Pike M, Sellke FW, Stegmann TJ, Udelson JE, and Rosengart TK. Clinical trials in coronary angiogenesis: issues, problems, consensus: an expert panel summary. *Circulation* 102: E73–E86, 2000.
100. Slavin J. Fibroblast growth factors: at the heart of angiogenesis. *Cell Biol Int* 19: 431–444, 1995.
101. Stocker R and Keaney JF Jr. Role of oxidative modifications in atherosclerosis. *Physiol Rev* 84: 1381–1478, 2004.
102. Stockklauser-Farber K, Ballhausen T, Laufer A, and Rosen P. Influence of diabetes on cardiac nitric oxide synthase expression and activity. *Biochim Biophys Acta* 1535: 10–20, 2000.
103. Szabo C. PARP as a drug target for the therapy of diabetic cardiovascular dysfunction. *Drug News Perspect* 15: 197–205, 2002.
104. Tada H, Muramatsu I, Nakai T, Kigoshi S, and Miyabo S. Effects of chronic diabetes on the responsiveness to endothelin-1 and other agents of rat atria and thoracic aorta. *Gen Pharmacol* 25: 1221–1228, 1994.
105. Tamarat R, Silvestre JS, Huijberts M, Benessiano J, Ebrahimi TG, Duriez M, Wautier MP, Wautier JL, and Levy BI. Blockade of advanced glycation end-product formation restores ischemia-induced angiogenesis in diabetic mice. *Proc Natl Acad Sci U S A* 100: 8555–8560, 2003.
106. Taniyama Y, Morishita R, Hiraoka K, Aoki M, Nakagami H, Yamasaki K, Matsumoto K, Nakamura T, Kaneda Y, and Ogihara T. Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat diabetic hind limb ischemia model: molecular mechanisms of delayed angiogenesis in diabetes. *Circulation* 104: 2344–2350, 2001.
107. Thirunavukkarasu M, Penumathsa SV, Koneru S, Juhasz B, Zhan L, Otani H, Bagchi D, Das DK, and Maulik N. Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: role of nitric oxide, thioredoxin, and heme oxygenase. *Free Radic Biol Med* 43: 720–729, 2007.
108. Tio RA, Tan ES, Jessurun GA, Veegeer N, Jager PL, Slart RH, de Jong RM, Pruim J, Hospers GA, Willemsen AT, de Jongste MJ, van Boven AJ, van Veldhuisen DJ, and Zijlstra F. PET for evaluation of differential myocardial perfusion dynamics after VEGF gene therapy and laser therapy in end-stage coronary artery disease. *J Nucl Med* 45: 1437–1443, 2004.
109. Tofukuji M, Metais C, Li J, Franklin A, Simons M, and Sellke FW. Myocardial VEGF expression after cardiopulmonary bypass and cardioplegia. *Circulation* 98: II242–II246; discussion II247–II248, 1998.
110. Toyota E, Matsunaga T, and Chilian WM. Myocardial angiogenesis. *Mol Cell Biochem* 264: 35–44, 2004.
111. Tyagi SC. Vasculogenesis and angiogenesis: extracellular matrix remodeling in coronary collateral arteries and the ischemic heart. *J Cell Biochem* 65: 388–394, 1997.
112. Uhlmann S, Friedrichs U, Eichler W, Hoffmann S, and Wiedemann P. Direct measurement of VEGF-induced nitric oxide production by choroidal endothelial cells. *Microvasc Res* 62: 179–189, 2001.
113. Unger EF, Goncalves L, Epstein SE, Chew EY, Trapnell CB, Cannon RO 3rd, and Quyyumi AA. Effects of a single intracoronary injection of basic fibroblast growth factor in stable angina pectoris. *Am J Cardiol* 85: 1414–1419, 2000.
114. Urbich C, Dernbach E, Zeiher AM, and Dimmeler S. Double-edged role of statins in angiogenesis signaling. *Circ Res* 90: 737–744, 2002.
115. Vale PR, Losordo DW, Milliken CE, McDonald MC, Gravelin LM, Curry CM, Esakof DD, Maysky M, Symes JF, and Isner JM. Randomized, single-blind, placebo-controlled pilot study of catheter-based myocardial gene transfer for therapeutic angiogenesis using left ventricular electromechanical mapping in patients with chronic myocardial ischemia. *Circulation* 103: 2138–2143, 2001.
116. van Etten RW, de Koning EJ, Verhaar MC, Gaillard CA, and Rabelink TJ. Impaired NO-dependent vasodilation in patients with type II (non-insulin-dependent) diabetes mellitus is restored by acute administration of folate. *Diabetologia* 45: 1004–1010, 2002.
117. Voisine P, Bianchi C, Khan TA, Ruel M, Xu SH, Feng J, Li J, Malik T, Rosinberg A, and Sellke FW. Normalization of coronary microvascular reactivity and improvement in myocardial perfusion by surgical vascular endothelial growth factor therapy combined with oral supplementation of L-arginine in a porcine model of endothelial dysfunction. *J Thorac Cardiovasc Surg* 129: 1414–1420, 2005.
118. Voisine P, Bianchi C, Ruel M, Malik T, Rosinberg A, Feng J, Khan TA, Xu SH, Sandmeyer J, Laham RJ, and Sellke FW. Inhibition of the cardiac angiogenic response to exogenous vascular endothelial growth factor. *Surgery* 136: 407–415, 2004.
119. Voisine P, Li J, Bianchi C, Khan TA, Ruel M, Xu SH, Feng J, Rosinberg A, Malik T, Nakai Y, and Sellke FW. Effects of L-arginine on fibroblast growth factor 2-induced angiogenesis in a model of endothelial dysfunction. *Circulation* 112: I202–I207, 2005.
120. Wang SY, Friedman M, Johnson RG, Weintraub RM, and Sellke FW. Adrenergic regulation of coronary microcirculation after extracorporeal circulation and crystalloid cardioplegia. *Am J Physiol* 267: H2462–H2470, 1994.

121. Watts GF, O'Brien SF, Silvester W, and Millar JA. Impaired endothelium-dependent and independent dilatation of forearm resistance arteries in men with diet-treated non-insulin-dependent diabetes: role of dyslipidaemia. *Clin Sci (Lond)* 91: 567–573, 1996.
122. Wautier JL and Schmidt AM. Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 95: 233–238, 2004.
123. Weihrauch D, Lohr NL, Mraovic B, Ludwig LM, Chilian WM, Pagel PS, Warltier DC, and Kersten JR. Chronic hyperglycemia attenuates coronary collateral development and impairs proliferative properties of myocardial interstitial fluid by production of angiotensin. *Circulation* 109: 2343–2348, 2004.
124. Weis M, Heeschen C, Glassford AJ, and Cooke JP. Statins have biphasic effects on angiogenesis. *Circulation* 105: 739–745, 2002.
125. White MF. Insulin signaling in health and disease. *Science* 302: 1710–1711, 2003.
126. Williams SB, Cusco JA, Roddy MA, Johnstone MT, and Creager MA. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 27: 567–574, 1996.
127. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, and Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 407: 242–248, 2000.
128. Yarom R, Zirkin H, Stammner G, and Rose AG. Human coronary microvessels in diabetes and ischaemia: morphometric study of autopsy material. *J Pathol* 166: 265–270, 1992.
129. Zhang L, Zalewski A, Liu Y, Mazurek T, Cowan S, Martin JL, Hofmann SM, Vlassara H, and Shi Y. Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. *Circulation* 108: 472–478, 2003.
130. Zhang X, Zhao SP, Li XP, Gao M, and Zhou QC. Endothelium-dependent and -independent functions are impaired in patients with coronary heart disease. *Atherosclerosis* 149: 19–24, 2000.
131. Zhang Y, Li J, Partovian C, Sellke FW, and Simons M. Syndecan-4 modulates basic fibroblast growth factor 2 signaling in vivo. *Am J Physiol Heart Circ Physiol* 284: H2078–H2082, 2003.
132. Ziche M. Role of nitric oxide in the angiogenesis of avascular tissue. *Osteoarthritis Cartilage* 7: 403–405, 1999.
133. Ziche M, Morbidelli L, Choudhuri R, Zhang HT, Donnini S, Granger HJ, and Bicknell R. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. *J Clin Invest* 99: 2625–2634, 1997.
134. Ziche M, Morbidelli L, Masini E, Amerini S, Granger HJ, Maggi CA, Geppetti P, and Ledda F. Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. *J Clin Invest* 94: 2036–2044, 1994.

Address correspondence to:

Frank W. Sellke, M.D.

Division of Cardiothoracic Surgery

Beth Israel Deaconess Medical Center

110 Francis Street Suite 2A

Boston, MA 02215

E-mail: fsellke@caregroup.harvard.edu

Date of first submission to ARS Central, January 12, 2009; date of acceptance, January 17, 2009.

This article has been cited by:

1. Luciano Tavares Angelo Cintra, Aguinaldo Cândido Silva Facundo, Mariane Maffei Azuma, Dóris Hissako Sumida, Rafael Dias Astolphi, Suely Regina Mogami Bomfim, Luís Gustavo Narciso, João Eduardo Gomes-Filho. 2012. Pulpal and periodontal diseases increase triglyceride levels in diabetic rats. *Clinical Oral Investigations* . [[CrossRef](#)]
2. Hassan I.H. El-Sayyad, Mohamed S. Al-Haggar, Heba A. El-Ghawet, Iman H. Bakr. 2012. Cardiomyopathy and angiogenesis defects of Wistar rat fetuses of diabetic and hypercholesterolemic mothers. *Nutrition* **28**:7-8, e33-e43. [[CrossRef](#)]
3. Antonio D. Lassaletta, Louis M. Chu, Frank W. Sellke. 2012. Effects of alcohol on pericardial adhesion formation in hypercholesterolemic swine. *The Journal of Thoracic and Cardiovascular Surgery* **143**:4, 953-959. [[CrossRef](#)]
4. Andrea Caporali, Costanza Emanuelli. 2012. MicroRNAs in Postischemic Vascular Repair. *Cardiology Research and Practice* **2012**, 1-7. [[CrossRef](#)]
5. Michael P Robich, Louis M Chu, Shizu Oyamada, Neel R Sodha, Frank W Sellke. 2011. Myocardial therapeutic angiogenesis: a review of the state of development and future obstacles. *Expert Review of Cardiovascular Therapy* **9**:11, 1469-1479. [[CrossRef](#)]
6. Antonio D. Lassaletta, Louis M. Chu, Frank W. Sellke. 2011. Therapeutic neovascularization for coronary disease: current state and future prospects. *Basic Research in Cardiology* . [[CrossRef](#)]
7. Michael P. Robich, Robert M. Osipov, Louis M. Chu, Jun Feng, Thomas A. Burgess, Shizu Oyamada, Richard T. Clements, Roger J. Laham, Frank W. Sellke. 2010. Temporal and Spatial Changes in Collateral Formation and Function During Chronic Myocardial Ischemia. *Journal of the American College of Surgeons* **211**:4, 470-480. [[CrossRef](#)]
8. D. NEIL GRANGER, STEPHEN F. RODRIGUES, ALPER YILDIRIM, ELENA Y. SENCHENKOVA. 2010. Microvascular Responses to Cardiovascular Risk Factors. *Microcirculation* **17**:3, 192-205. [[CrossRef](#)]