Nitric oxide release from the endothelium plays an important role in regulation of vascular tone, inhibition of both platelet and leukocyte aggregation and adhesion, and inhibition of cell proliferation. These properties suggest that the level of NO production by the endothelium may play a pivotal role in the regulation of vascular disease. Analysis using mass spectrometry has revealed that NO is produced by NOS from the terminal guanidino nitrogen of the precursor amino acid L-arginine. Thus, utilization of L-arginine and conversion to NO may establish a regulatory site in the development of endothelial dysfunction.

Endothelial dysfunction is characterized by defective endothelium-dependent relaxation, and some reviews regarding endothelial dysfunction in diabetes have been published. These reviews have focused on factors that might contribute to defective relaxation, some of which will not be addressed in detail in this review. The purpose of the present review is to summarize evidence that specifically supports either decreased NO production by diabetic vascular endothelium and/or impaired NO-mediated endothelium-dependent relaxation. Second, this review provides reasonable alternatives to explain some of the controversies in this research area. Third, since there is growing evidence that arginine appears to have some benefits for diabetes-associated abnormalities, this review summarizes the current state of knowledge of effects of acute and chronic administration of L-arginine on diabetes-induced endothelial dysfunction and discusses potential NO-dependent and -independent mechanisms whereby therapeutic intervention with L-arginine might benefit the diabetic endothelium.

Impaired Endothelium-Dependent Relaxation

Experimental Diabetes Mellitus

Decreases in endothelium-dependent relaxation are a common feature in both conduit and resistance arteries of chemically induced experimental diabetic animals (including rats, mice, rabbits, hamsters, and dogs). In two genetic models of IDDM, similar impaired relaxation has been documented in aorta and mesenteric arteries of diabetes-prone BB/Wor or BB/E rats and in aorta and mesenteric arteries of the diabetes-prone WBN/Kob rat.

Almost without exception, studies that have shown impaired endothelium-dependent relaxation have found normal relaxation to nitrovasodilators. These agents relax vascular smooth muscle by activating guanylate cyclase but, unlike NO, do not require the presence of the endothelium. Thus, the intrinsic property to activate vascular smooth muscle guanylate cyclase appears not to be altered by experimental diabetes.

Studies in experimental models of NIDDM are few and controversial. In the obese Zucker rat, no alteration in endothelium-dependent relaxation has been observed in intestinal microvessels, whereas increased reactivity has been observed in aorta. In contrast, decreased endothelium-dependent relaxation to acetylcholine but not to A23187 was seen in aorta in male (but not female) JCR:LA-corpulent rats. Little is known regarding the nature of endothelial dysfunction in these models.

Conclusion

Collectively, these studies suggest that endothelial dysfunction (1) is not unique to chemically induced experimental models of IDDM, (2) is found in both conduit and resistance arteries, and (3) cannot be explained by an intrinsic change in reactivity to NO or guanylate cyclase reactivity. Studies conducted in experimental models of NIDDM are few and provide no clear consensus regarding this issue.

Human Diabetes Mellitus

The first evidence of endothelial dysfunction in humans was reported in penile corpora cavernosa of IDDM and NIDDM patients. Impaired endothelium-dependent relaxation of conduit blood vessels has been confirmed in both IDDM and NIDDM patients evaluated in vivo or in isolated arteries in vitro. A few studies in IDDM or NIDDM patients report no defect. One study of resistance vessels derived from NIDDM patients and evaluated in vitro showed enhanced reactivity to acetylcholine. Evaluation of the latter study is compromised because the control patients selected exhibited arterial occlusive disease, hypercholesterolemia,
Selected Abbreviations and Acronyms

EDHF = endothelium-derived hyperpolarizing factor
IDDM = insulin-dependent diabetes mellitus
NIDDM = non–insulin-dependent diabetes mellitus
NO(S) = nitric oxide (synthase)
ODQ = 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one
SOD = superoxide dismutase
TEA = tetraethylammonium

and other nondiabetic diseases. Also, patients using various cardiovascular medications were not excluded from the study. Several investigators have observed normal relaxation to nitrovasodilators in diabetic patients, 42–47,51,55–60 In contrast, other investigators have noted attenuated responses to nitrovasodilators in IDDM diabetic patients 54,61–63 and in NIDDM diabetic patients.46–50,64

Conclusion. The majority of studies have demonstrated impaired endothelium-dependent relaxation. In contrast, there is not sufficient consensus on whether vascular smooth muscle reactivity to NO is compromised in human diabetes mellitus.

Importance of Patient Screening and Other Complicating Factors

One possible explanation of this variability relates to primary nitrate tolerance, since responses to nitroglycerin but not sodium nitroprusside were diminished in diabetic patients.65 Alternatively, one of these studies noted no difference in reactivity to nitroglycerin after correction for differences in baseline diameter.51 Furthermore, diabetic patients with microalbuminuria exhibited impaired dilation to isosorbide dinitrate, while in diabetic patients without microalbuminuria, dilation was unaltered.66 In this regard, dilation in response to carbachol was normal in diabetic patients but was blunted by an NOS inhibitor in both control and diabetic patients without microalbuminuria but not in diabetic patients with microalbuminuria.56 Thus, there may be variable mechanisms of impaired vascular reactivity to nitrovasodilators among different diabetic patient subpopulations.

There are several concerns regarding critical evaluation of the data obtained in diabetic patients. Whereas some investigators have carefully eliminated patients taking medications (eg, vasoactive and cardiovascular drugs) and have limited intake of caffeine or alcohol before evaluation, other investigators have either not provided information regarding patient selection or have ignored these potential influences. Some studies have included patients with histories of smoking (an independent risk factor for endothelial dysfunction) or have had only limited smoking just before the study.

Most studies have used a mixed-gender patient population. Thus, the influence of gender-related differences has not been isolated. Indeed, it is widely known that diabetes-induced complications are enhanced in the female population. A recent study has revealed diminished reactive hyperemia (an endothelium-related phenomenon) in young female IDDM patients compared with age-matched male subjects.67 Furthermore, inclusion of postpuberty female patients using oral contraceptive agents has been acknowledged in some stud-
ies, 61,62 but the significance of inclusion of this subpopulation has not been addressed. In studies using male subjects only, endothelial function was either impaired 46–47,49–50 or unchanged.54,55,57

In studies of vascular reactivity using NIDDM patients, it was acknowledged that many subjects were receiving hypoglycemic drugs or insulin at the time of evaluation, 57–59,51,52,64,68 Oral hypoglycemic agents such as glibenclamide can impair dilation mediated by NO-mediated K\textsubscript{ATP} channel activation.69 Thus, the effects of this medication on NO-mediated relaxation have not been uniformly excluded. One study showed no difference in endothelium-dependent relaxation between patients taking oral hypoglycemic agents and those controlling the disease by diet alone.64 In one study in which hypoglycemic medication was removed for a finite period before evaluation, there was diminished reactivity to acetylcholine but not to nitroprusside.53

Endothelial function in patients has been determined in vivo using either strain-gauge plethysmography or Doppler techniques, predominately in brachial/forearm protocols. A few have evaluated dilation in the femoral artery 53,63,64 or coronary arteries.41 While there is important value in examining reactivity in vivo under ambient conditions, there are several significant complicating factors that make the in vivo delineation of mechanisms difficult. For example, one investigator found that endothelial dysfunction correlated with serum triglyceride and lower HDL cholesterol levels.50 Furthermore, ambient glycated proteins may diminish endothelium-dependent relaxation studied in vitro,70,71 although a recent in vitro study in canine conduit and resistance artery preparations could not confirm this effect.72

Conclusion. Patient selection and screening have the potential to explain differences in findings obtained in clinical studies. The potential influence of gender, presence of microalbuminuria, and use of medications, including oral contraceptives and hypoglycemic agents, need to be rigorously evaluated in clinical studies. Also, the influence of ambient glucose concentration, glycated proteins, and lipids on endothelial function needs to be considered in clinical evaluations.

Confounding Effects of Ambient Glucose Concentration

Ambient plasma glucose concentration could be a confounding factor in vivo as well. Some studies in experimental animal models have noted acute vasodilation (ie, seconds to minutes) in response to concurrent elevation in glucose concentration.73–75 In contrast, short-term exposure (ie, minutes to several hours) to elevated glucose concentrations in vitro or topical application in situ impairs basal NO tone  65 and agonist-stimulated endothelium-dependent relaxation.77–81 Interestingly, 24-hour infusion of glucose in normal subjects did not impair relaxation.82 Because multiple agonists were used in the latter study, the significance of masking by crossover effects of individual drugs cannot be excluded in the interpretation of this data. In contrast, evaluation during an acute bolus of glucose administration (ie, during a glucose tolerance test) revealed decreased endothelium-dependent relaxation.83

Confounding Effects of Other Complicating Factors

Several studies have included patients with histories of smoking (an independent risk factor for endothelial dysfunction) or have included only limited smoking just before the study. Some studies have included patients with histories of smoking (an independent risk factor for endothelial dysfunction) or have included only limited smoking just before the study. Some studies have included patients with histories of smoking (an independent risk factor for endothelial dysfunction) or have included only limited smoking just before the study. Some studies have included patients with histories of smoking (an independent risk factor for endothelial dysfunction) or have included only limited smoking just before the study.
An immediate acute effect of elevated glucose concentration is a marked increase in basal \([Ca^{2+}]\), and NO production in isolated human endothelial cells.\(^{64}\) [Ca\(^{2+}\)], also increases rapidly (within minutes) in the presence of elevated glucose concentration in isolated bovine aortic endothelial cells (G.M.P., unpublished observation, 1997). This effect may persist for several hours after exposure to elevated glucose concentration.\(^{65}\) In contrast, after 24 hours of exposure of bovine aortic endothelial cells to elevated glucose and subsequent analysis under normal glucose conditions, basal [Ca\(^{2+}\)], and NO production returned to normal, whereas bradykinin-stimulated [Ca\(^{2+}\)], and NO production was reduced.\(^{66}\) Similarly, impaired agonist-stimulated [Ca\(^{2+}\)], and NO production have been observed after exposure of rat or porcine endothelial cells to elevated glucose concentration.\(^{87,88}\)

To circumvent this potential limitation, one study using the euglycemic insulin clamp protocol\(^{66}\) revealed that endothelial function was diminished. Also, analysis in vitro of human gluteal resistance arteries under normoglycemic conditions also revealed diminished endothelium-dependent relaxation.\(^{45}\) The latter study suggested an intrinsic defect in endothelium-dependent relaxation in IDDM that is independent of direct effects of concurrent ambient glucose concentrations in vivo. In contrast, another study showed that relaxation to carbachol was unchanged in IDDM patients under euglycemic conditions produced by insulin infusions.\(^{86}\) Interestingly, a defect in NO synthesis was still suggested in patients with microalbuminuria based on the lack of response to L-NMMA (L-N-Monomethyl-L-arginine).

**Conclusion.** The confounding effects of ambient glucose concentration on endothelial function in studies performed in patients and in experimental diabetes in vivo should be taken into account when glucose concentration is not normalized. Unfortunately, controlling for this contingency may be difficult, particularly in clinical studies. Clearly, there are important direct stimulatory and inhibitory effects of elevated glucose concentration on vascular reactivity that may be manifested at different times subsequent to rises in glucose concentration.

**Paradoxical Findings on Endothelial Function: Role of Disease Duration**

Few studies have examined the temporal nature of the onset of endothelial dysfunction in experimental diabetes. Most have shown a progressive worsening of dysfunction that appears to plateau at some finite point in time. Dysfunction has been reported at as early as 1 week of diabetes in rat intestinal arterioles,\(^{70}\) after 2 weeks in hindquarters but not in mesenteric or renal arteries,\(^{89}\) after 3 weeks in cremaster muscle arterioles,\(^{90}\) after 4 to 6 weeks in mesenteric arterioles,\(^{25,91}\) and after 4 weeks in aorta.\(^{78}\) Thus, lack of endothelial dysfunction of diabetic rat aorta at 2 to 3 weeks of disease\(^{92}\) could be explained by the short period of time studied. It is important to note that the onset of endothelial dysfunction may vary widely among individual vascular beds and/or the severity of the diabetic model used in any given study.

Interestingly, there are paradoxical reports of normal endothelium-dependent relaxation in aorta after >12 weeks of diabetes\(^{93–95}\) and after 15 to 17 weeks of diabetes in perfused mesenteric preparations.\(^{96}\) The reasons for these disparate observations compared with other findings are unclear. Potential explanations might include the use of helical strips,\(^{93}\) diabetes-induced hypersensitivity to phenylephrine and evaluation only in indomethacin-treated preparations,\(^{97}\) use of a low dose of streptozotocin,\(^{94}\) or the potential influence of masking by crossover effects due to multiple drug challenges.\(^{95}\)

There is clinical and experimental evidence showing augmented blood flow at early stages of diabetes.\(^{97}\) It is not at all clear whether this increased blood flow reflects increased NO-specific endothelium-dependent dilation. One possibility that needs to be examined is whether the diabetes-induced decreases in 2,3-diphosphoglyceric acid levels in red blood cells,\(^{98}\) which regulate oxygen release from hemoglobin, lead to a “hypoxic-like” environment.\(^{99}\) Decreases in tissue ATP concentration could result in increased blood flow due to hypoxic-induced dilation in compensation for reduced oxygen delivery to tissue. Indeed, myocardial ATP concentration is reduced in diabetes but is rapidly replenished within minutes of perfusion in vitro with oxygenated blood-free salt solutions.\(^{100}\) Furthermore, in situ analysis using microelectrodes reveals diminished oxygen tension in aorta of alloxan-diabetic rabbits.\(^{101}\) Thus, increase in tissue blood flow at the early stages of diabetes may be due to hypoxic vasodilation. Alternatively, increased blood flow may be a direct response to acute or short-term hyperglycemia, since coronary blood flow is increased with glucose infusions in isolated hearts in the presence of indomethacin.\(^{75}\)

At least one study has noted augmented endothelium-dependent relaxation in indomethacin-treated rat renal arteries at an early stage of diabetes.\(^{102}\) Because endothelium-dependent relaxation is reduced in renal arteries of diabetic rats of longer disease duration,\(^{103}\) this observation raises the possibility that diabetes induces biphasic effects on endothelium-dependent relaxation. Thus, an early increase in blood flow may be followed by a transition state to impaired relaxation. In support of this hypothesis, one study noted an increase in endothelium-dependent relaxation of mesenteric arteries to both acetylcholine and bradykinin at 6 weeks of diabetes that reverts back to normal after 12 weeks.\(^{104}\) This enhanced relaxation could not be accounted for by intrinsic changes in smooth muscle reactivity because responses to sodium nitroprusside were normal.

It remains to be resolved in temporal studies using various preparations whether the enhanced endothelium-dependent relaxation at early stages might be due to diabetes-induced increases in synthesis of vasodilator prostaglandins, EDHF, or NO, or a combination of any of these endothelium-derived factors. In this regard, studies conducted in mesenteric\(^{104}\) and renal\(^{102}\) arteries in the presence of indomethacin suggest that vasoactive prostanoids do not contribute to enhanced endothelium-dependent relaxation, leaving EDHF and NO as candidate factors.

**Conclusion.** Time of evaluation after onset of disease may be critical to demonstrating endothelial dysfunction. Indeed,
Role of Prostanoids in Endothelium-Dependent Dilation in Diabetes

Impaired endothelium-dependent relaxation in diabetes cannot always be assumed to be mediated by a reduction in NO activity or synthesis, since some vasodilators also release prostaglandins. Changes in prostaglandin synthesis may alter NO production or reactivity to NO. Because diabetes alters the reactivity to prostanooids and may either increase or decrease prostacyclin production depending on the artery chosen,\(^\text{105}\) this issue needs to be resolved, especially at various stages of disease and in individual blood vessel preparations.

Simultaneous enhancement and release of vasoconstrictor prostaglandins may explain some instances of impaired endothelium-dependent relaxation. Indeed, in aorta, pial artery, and mesenteric artery, relaxation is either normal\(^\text{104}\) or is normalized in the presence of indomethacin or thromboxane receptor antagonists.\(^\text{11,107}\) In contrast, endothelial function was normal in coronary arteries of alloxan-diabetic dogs, but dysfunction was unmasked in the presence of inhibitors of cyclooxygenase,\(^\text{11,107}\) suggesting enhanced compensatory increases in vasodilator prostanooid release. Furthermore, increases in production of the vasodilator prostacyclin have been reported in perfused mesenteric beds of 3-week diabetic rats.\(^\text{108}\) While not directly examined, this might provide an alternative explanation for one of the early reports showing augmented relaxation to the agonist histamine in mesenteric arteries of diabetic rats\(^\text{11,107}\) or the observation of normalized improvement in endothelial function after evaluation under conditions of cyclooxygenase blockade or thromboxane antagonism does not alter relaxation in basilar artery\(^\text{110}\) but restores relaxation of pial arteries taken from the streptozotocin-diabetic rat model.\(^\text{21}\) These observations suggest important regional differences, although one cannot exclude the possible contribution of the superimposition of the variable in the duration of disease, which was 4 to 5 months in the basilar artery study versus 2.5 to 3.5 months for the pial artery study. In another model of alloxan-diabetic dogs in which duration of disease was held constant, inclusion of indomethacin or ibuprofen unmasked a defect of endothelium-dependent relaxation in coronary artery\(^\text{111}\) but not renal artery.\(^\text{111}\) Collectively, these studies suggest important regional differences in the mechanism of endothelial dysfunction.

Many studies using either rat conduit arteries\(^\text{9,14,16–18,112–114}\) or rat resistance arteries\(^\text{25,26,91,103,115–117}\) have indicated no improvement in endothelial function after evaluation under conditions of cyclooxygenase blockade or thromboxane receptor antagonism. Similar interventions have failed to modify defective relaxation in aorta of the genetic diabetic BB rat\(^\text{13}\) and in coronary arteries of alloxan-diabetic dogs.\(^\text{20}\)

The effect of prostanooids on endothelial function in human diabetes has not been routinely evaluated. Prior treatment with indomethacin in vitro normalized impaired endothelium-dependent relaxation in cutaneous arteries of gestational diabetes.\(^\text{118}\) In contrast, in one human study in which all patients received aspirin before evaluation,\(^\text{44}\) the authors concluded that exogenous prostanooid synthesis cannot account for the endothelial dysfunction in IDDM.

Conclusion. There exist some potentially important regional differences in the role of prostanooids in contributing to altered endothelium-dependent dilation in diabetes mellitus, although this cannot be easily predicted among various conduit versus resistance vessels. Nevertheless, there is clear and ample evidence to suggest that alterations in prostanooid production may not always account for and/or may not be obligatory for impaired endothelial function in diabetes.

NO-Dependent or -Independent Endothelial Dysfunction

Implicit in all of these studies is the assumption that endothelium-dependent relaxation in both control and diabetic blood vessels is exclusively mediated via NO. This assumption is hazardous because endothelium-dependent relaxation to certain agonists and in certain arteries appears to be mediated in part by vasodilator prostanooids or by an EDHF.\(^\text{119}\) The entity of EDHF is not known with complete certainty, but at least one EDHF is believed to be an epoxide of arachidonic acid that is formed by a cytochrome \(P450\)-derived monoxygenase.\(^\text{120}\)

EDHF appears to activate \(K^+\) channels, especially calcium-activated \(K^+\) channels.\(^\text{119}\) The contribution of \(K_{\text{ATP}}\) channels to relaxation in diabetes is uncertain and should also be considered. A few studies have noted diminished responses to \(K_{\text{ATP}}\) channel openers in diabetic rat aorta\(^\text{11,121,122}\) and basilar artery.\(^\text{123}\) One study in the aorta of WBN/Kob rat showed no alteration in relaxation to the \(K_{\text{ATP}}\) channel agonist cromakalim.\(^\text{124}\) In contrast, others have observed a paradoxically enhanced response in dog coronaries to aprikalim, albeit in short-term diabetes.\(^\text{125}\) In normal arteries, it is generally believed that the component of EDHF versus NO that contributes to total relaxation increases with decreasing vessel size. The observation that diabetes-induced endothelial dysfunction can occur in both conduit and resistance arteries despite cyclooxygenase blockade suggests that this dysfunction could be explained by defects in EDHF or by deficits in NO synthesis unrelated to or in addition to changes in EDHF or prostanooids.

Two studies in rat aorta reveal that endothelial dysfunction persists despite pretreatment with TEA (to inhibit calcium-activated \(K^+\) channels), suggesting that defects in EDHF may not be operative.\(^\text{126,127}\) Alternatively, a recent study in perfused kidney indicates that endothelium-dependent relaxation in control kidney arises from both NO and EDHF, whereas relaxation in diabetic kidney arises from NO, EDHF, and prostanooids.\(^\text{128}\)

Few studies have examined perturbations in membrane polarization in diabetic vascular tissue. Membrane hyperpolarization is known to occur in gastric gland of 2- to 3-day diabetic rabbits\(^\text{129}\) and in endothelial cells from human sub-
jects with gestational diabetes.\textsuperscript{130} One study showed normal resting membrane potential but diminished hyperpolarization in the response of diabetic mesenteric artery to acetylcholine\textsuperscript{113} despite unaltered hyperpolarization in response to the K\textsuperscript{+} channel agonist pinacidil. This report conducted in the presence of NOS and cyclooxygenase inhibitors suggested diminished endothelium-dependent, TEA-sensitive hyperpolarization and relaxation.

**Conclusion.** Endothelial dysfunction in some cases of diabetes and in certain blood vessel types may arise from deficits in EDHF, but it is also clear that endothelial dysfunction can also occur despite blockade of EDHF and prostanoid synthesis and action, suggesting a role for deficits of endothelium-derived NO. It is possible that previous studies may need to be carefully reevaluated, since alternative compensatory pathways including cytochrome P450–derived EDHF may be activated or inactivated, which could mask impaired endothelium-dependent relaxation or compensate for defective NO synthesis. It should be emphasized that parallel compensatory pathways may be important at certain stages but perhaps not at all stages of the disease.

**Evidence Supporting Altered NO Production From Diabetic Endothelium**

**Functional Studies in the Presence of SOD**

NO activity is known to be reduced by chemical interaction and destruction by superoxide anion radicals. Several investigators have noted improved relaxation after acute incubation with SOD.\textsuperscript{114,125,126,133,137,138} This issue has been discussed in detail in a previous review.\textsuperscript{7} Nevertheless, these studies are consistent with increased destruction of NO and decreased bioactivity of NO in diabetes, but they do not give adequate direct information regarding perturbations in NO production.

Currently, there have been no studies that have directly determined NO synthesis or release from diabetic endothelium. Only one laboratory has examined intraluminal release of NO activity from perfused diabetic rat aorta donor segments using the bioassay technique.\textsuperscript{133} Accordingly, luminally released basal NO activity in perfused diabetic rat aorta was normal, but addition of SOD caused a larger incremental increase in relaxation of the bioassay detector when the diabetic donor segment was used. This suggested the increased release of superoxide anion radicals from diabetic rat endothelium. In contrast, acetylcholine-stimulated endothelium-derived relaxing factor/NO bioactivity from perfused diabetic rat aorta was diminished but also normalized by perfusion with SOD.\textsuperscript{114}

**Conclusion.** Several studies support the concept that IDDM decreases NO bioactivity. This may be due in part to enhanced destruction of NO by increased superoxide synthesis.

**Indirect Evidence Using Guanylate Cyclase Inhibitors**

Use of inhibitors of NOS or NO reactivity (eg, hemoglobin or methylene blue) has given indirect information regarding the contribution of NO to basal tone and agonist-induced relaxation in control versus diseased blood vessels. Many studies that have used removal of endothelium or NOS inhibitors or hemoglobin, which increase the precontracted tone of arteries, reveal decreased basal NO activity in both diabetic conduit\textsuperscript{8,9,134–140} and resistance\textsuperscript{125,135,137,140–145} arteries under both in vitro and in vivo conditions. Two studies noted enhanced contraction to methylene blue in control versus diabetic rat aorta ring preparations, suggesting diminished basal NO in diabetic arteries.\textsuperscript{16,131} The use of methylene blue alone is inadequate because of the known effects of methylene blue to stimulate superoxide anion radical or inhibit NOS.\textsuperscript{146,147} One study using a new highly selective inhibitor of guanylate cyclase, ODQ, revealed that ODQ completely inhibits acetylcholine-induced relaxation in control rat aorta and inhibits relaxation in diabetic rat aorta by 80%.\textsuperscript{148} This suggests that almost all of the relaxation in both groups is mediated by cGMP activation, implicating NO as the EDRF. Also, the small ODQ-resistant component of relaxation in diabetic rat aorta leaves open the possibility of some other unknown factor that appears unrelated to vasodilator prostanoids, H\textsubscript{2}O\textsubscript{2}, or a TEA-sensitive EDHF.

**Conclusion.** Overall, these studies suggest diminished basal NO production in diabetes.

**Evidence Using NO Trapping Agents**

Basal NO activity in rat aorta has also been assessed using either a small-molecular-weight, iron-thiol-based NO scavenger\textsuperscript{149} or a nitronyl nitroxide trapping agent.\textsuperscript{148} These agents are different in that they do not alter NOS activity but react with NO after its release. These probes were effective in scavenging all basal NO based on similar tension responses after removal of endothelium or by using NOS inhibitors and confirm a smaller basal NO activity in diabetic rat aorta. This NO scavenger–sensitive basal NO activity was augmented by 1-arginine in diabetic but not control aorta.\textsuperscript{149}

In contrast, despite nearly complete inhibition of acetylcholine-mediated relaxation by both control and diabetic rat aorta by NOS inhibitors, use of a nitronyl nitroxide revealed an NO scavenger–resistant component of agonist-stimulated relaxation that is greater in diabetic than control arteries.\textsuperscript{148} This resistant component of endothelium-dependent relaxation was sensitive to NOS inhibitor but was insensitive to indomethacin, TEA, or catalase, suggesting that this additional EDRF is likely not EDHF, prostanoids, or H\textsubscript{2}O\textsubscript{2}. A significant portion of this resistant component was eliminated using ODQ, suggesting that this EDRF is an activator of guanylate cyclase. Current research continues to attempt to identify whether diabetic arteries produce an additional vasooactive product that is derived from the NOS reaction but that may not necessarily be a free NO radical.

**Measurement of NO by cGMP Generation**

Information regarding potential deficits in NO synthesis have also been derived from measurement of cGMP in vascular tissue. Although one study showed no significant alteration in basal and acetylcholine-stimulated cGMP in alloxan-diabetic aorta,\textsuperscript{150} an earlier study in the same animal model showed that diabetes decreased both basal and acetylcholine-stimulated cGMP.\textsuperscript{59} The latter study differed only in that duration of disease was longer and that a preconstrictor agonist was
used in the assays. Similar decreases in acetylcholine-stimulated cGMP production have been observed in rat and rabbit aorta and mesenteric arteries, \(^{12,19,131,152}\) in canine coronary arteries, \(^{107}\) and in isolated rat glomeruli. \(^{153,154}\) In contrast, cGMP in response to nitroprusside was diminished in isolated glomeruli from diabetic rats. \(^{153}\) In the presence of the phosphodiesterase inhibitor IBMX and after stimulation with nitroprusside, cGMP production was not altered by diabetes in rat aorta \(^{152}\) or glomeruli. \(^{154}\)

There is no apparent intrinsic change in either guanylate cyclase or phosphodiesterase activity of vascular smooth muscle to account for defective cGMP production in diabetic blood vessels. The evidence suggests decreased basal and agonist-stimulated NO bioactivity in experimental diabetes mellitus.

**Measurement of NO or NO By-products**

To our knowledge, there has been no direct measurement of NO from diabetic arteries using NO electrodes or an NO analyzer. According to indirect measurements of the NO-based by-products nitrate and nitrite, levels of either nitrite or nitrate + nitrite in urine of diabetic rats were increased. \(^{16,142,155-157}\) Streptozotocin, the agent frequently used to produce experimental diabetes, is also a known NO donor compound. \(^{162}\) This property could potentially contribute to enhanced urinary nitrate/nitrite levels at early stages of diabetes. This limitation is likely not a problem for samples taken several weeks after the administration of streptozotocin. Furthermore, the increased urinary nitrate + nitrite might simply be secondary to hyperfiltration. Although some studies have controlled for nitrate/nitrite derived from food during diabetes-induced hyperphagia, most have not controlled for variation in nitrate consumption derived from drinking water due to diabetes-induced increases in water consumption or for NO released simply because of the mechanical stimuli of increased renal blood flow and filtration due to excess volume disposal as a consequence of diabetes. Thus, the relationship between urinary nitrate/nitrite concentration and vascular endothelium production of NO must be understood in the context of these significant limitations.

Some studies have reported increases in both urine and plasma nitrate + nitrite concentration in diabetic animals. \(^{157-159,161}\) In contrast, levels of plasma nitrate \(^{163}\) and of cGMP \(^{164}\) were normal in diabetic patients. Although nitrate levels were normal, \(^{163}\) exhaled NO level was decreased by 30% but did not reach statistical significance because of the small sample size. Infusion of L-NMMA caused a greater decrease in exhaled NO in healthy versus diabetic patients, although nitrate levels did not change, suggesting decreased NO synthesis. Nevertheless, changes in plasma NO do not provide concrete information regarding the location and source of NO production.

**Conclusion.** Measurement of nitrate/nitrite in plasma and urine in experimental diabetes supports increased NO production. Because the source of this increased nitrate and nitrite is not easily ascertained, conclusions regarding endothelial cell NO production based on these measures alone are hazardous and must be taken as confirmation of other derived parameters.

**Measurement of NOS Protein, mRNA, or Activity**

There is little information available concerning measurement of endothelial cell NOS protein content or mRNA in diabetes mellitus. Diaphorase staining and immunohistochemical staining suggest reduced NOS activity in sympathetic autonomic ganglia \(^{165}\) and macula densa \(^{166}\) of experimental diabetic rats. Another study revealed a decrease in mRNA and content of neuronal NOS (nNOS) in diabetic penile tissue. \(^{167}\) Interestingly, one study in diabetic rat heart homogenates noted increased NOS activity (believed to be largely constitutive NOS [cNOS]) determined by arginine to citrulline conversion along with increased cNOS mRNA and protein. \(^{26}\) There was no detectable inducible NOS (iNOS), although induction of iNOS was previously reported in placenta of gestational diabetic patients. \(^{168}\) In rat renal tissue at 1 week of diabetes, mRNA and protein for cNOS, iNOS, and NOS were all increased in cortex but unchanged in medulla. \(^{169}\)

Consistent with these reports are increases in mRNA and/or protein for cNOS after culture with elevated glucose concentrations in bovine aortic endothelial cells for 24 hours (G.M.P., unpublished observations, 1997) and in human aortic endothelial cells after 5 days. \(^{170}\) In contrast, longer-term culture under high glucose conditions of human umbilical vein endothelial cells revealed no alteration in either mRNA or protein for cNOS. \(^{171}\) This suggests either heterogeneity in the response of different endothelial cells or glucose concentration–dependent and/or exposure time–dependent differences.

In the diabetic heart study, the finding that normal or enhanced NOS activity occurs under acellular conditions despite concomitant decreases in endothelium-dependent relaxation indicates no intrinsic defect in NOS activity. This is consistent with elevations in mRNA for endothelial cell–derived NOS despite decreased NO production in endothelial cells cultured with elevated glucose for 24 hours. \(^{170}\)

In isolated endothelial cells from diabetic BB rat, \(^{172}\) nitrate/nitrite production in culture was diminished, as well as citrulline production from arginine, and occurred without changes in arginine uptake. The latter suggests no defect in the \(y^+\) transporter function in these cells. In contrast, alloxan diabetes enhanced CGMP production and increased both \(V_{\text{max}}\) and \(K_m\) for arginine in gastric glands. \(^{172}\) The significance of these observations to arginine content in vascular disease is unknown; however, it should be noted that these studies were conducted just 2 to 3 days after diabetes induction. Similar actions have been found by this same group in human fetal endothelial cells in gestational diabetes. \(^{130}\)

**Conclusion.** There are too few measurements of NOS activity, mRNA, and protein in the literature to formulate a consensus. There is some evidence of increased mRNA and protein despite impaired endothelial function. These observations suggest that NO synthesis might be regulated by factors present in the intact cell under conditions (eg, substrate provision or cofactor concentration) that may not be optimal for NOS enzyme activity (see “Discussion”).

**Arginine Deficiency and/or Substrate Utilization by NOS**

Plasma arginine concentration is decreased in diabetic rats \(^{26,138,159,173,174}\) and in some human diabetic patients. \(^{175,176}\) In
contrast, some reports involving a small number of patients have reported normal arginine levels. In streptozotocin-induced diabetes, decreases in plasma arginine and endothelial dysfunction are corrected by subsequent transplantation of either whole pancreas or pancreatic islets.

The reasons for decreased plasma arginine are unclear. One study showed that plasma arginine was decreased at 14 days. This was associated with a larger excretion of arginine with increases in nitrate/nitrite and cGMP, suggesting that lower plasma arginine results from increased renal NO production and/or increased elimination of arginine. Tissue levels of arginine might be decreased as the plasma arginine concentration in diabetic rats is near the $K_m$ for arginine transport into endothelial cells and the $K_m$ does not appear to change, at least in certain tissue and during early stages of diabetes.

There is a paucity of information regarding arginine content in diabetic tissue. Despite increased arginine content in gastric glands of 2- to 3-day allooxan-diabetic rabbits, the content of arginine in freshly isolated diabetic rat aorta is reduced. If arginine content is in fact diminished in various vascular tissue in diabetes, then replacement therapy should improve NO-mediated relaxation. One study showed that L-arginine partially improved, albeit modestly, basal and agonist-stimulated cGMP in diabetic glomeruli. Incubation in vitro with L-arginine but not D-arginine augments relaxation to acetylcholine in diabetic rat aorta. It is difficult to reconcile why one does not always observe diminished relaxation in all studies and to all endothelium-dependent vasodilators that also act via NO. Indeed, in the absence of arginine-supplemented buffer, receptor-independent, endothelium-dependent relaxation to the calcium ionophore A23187 in aorta that is normally blocked fully by NOS inhibitors is not diminished in either streptozotocin-induced or genetically prone BB diabetic rats.

Third, tissue arginine content in diabetic rat aorta is decreased despite the fact that samples were freshly extracted from animals (ie, without extended incubation in arginine-free media). Fourth, arginine content in isolated coronary endothelial cells of the diabetic BB rat was normal or even slightly elevated, yet these same cells demonstrated defective NO synthesis based on both nitrite release into media and arginine-to-citrulline conversion analysis. These studies also showed that NOS activity in cell homogenates was normal, suggesting impaired arginine utilization under intact cell conditions. Fifth, acetylcholine-stimulated relaxation of diabetic canine coronary arteries was decreased in vivo but not in vitro, and addition of L-arginine to diabetic canine coronary arteries in vitro did not enhance the diminished acetylcholine-stimulated cGMP production. In contrast, in other studies, intracoronary infusion of L-arginine infusion in diabetic dog hearts improved endothelium-dependent relaxation, and in situ application of L-arginine improved nerve blood flow in the rat.

These observations would argue against effects of L-arginine that are unique to the in vitro studies and unique to the rat model. Interestingly, effects in the diabetic dog in vivo occurred despite normal plasma arginine concentrations, suggesting that static plasma arginine levels need not be reduced for there to be improvement in endothelial function by L-arginine administration. Why this occurs is unknown. One possibility is that arginine transport might be reduced directly by elevated glycated proteins in the blood.

There are reports that appear not to support the concept of defects in L-arginine utilization. In one case, topical application of L-arginine apparently did not improve relaxation in basilar artery of streptozotocin-induced diabetic rats, in cheek pouch microcirculation of diabetic hamsters, or in mesenteric artery of BB diabetic rats. Additional information is needed to determine with total certainty whether these studies reflect important regional differences in the beneficial action of L-arginine on reversal of diabetic endothelial dysfunction because of differences in arginine concentration, duration of exposure, location of application, and duration of disease (see “Discussion”) and the potential for crossover effects due to multiple agonist challenges in the same preparation in at least two of these studies.

In addition, the ineffectiveness of L-arginine in mesenteric arteries of BB rats is inconclusive, since a short incubation period was used...
as well as a concentration (ie, 10 μmol/L) that is 20 times lower than the physiological concentration of arginine in rat plasma and 10 times lower than the $K_m$ of arginine transport into endothelial cells. Furthermore, the potential of masking by crossover effects due to multiple agonist administration has not been excluded in these studies.

One should not discount the possibility that progressive changes in pathiology may make endothelial dysfunction resistant to modification by L-arginine administration. Indeed, arginine has been shown to be beneficial in short-term but not long-term atherosclerosis. Indeed, L-arginine administration in vitro restores endothelium-dependent relaxation after 8 weeks of diabetes but not after 12 weeks of diabetes in aorta of Lewis-strain diabetic rats. It may be noteworthy to consider the possibility that the negative results of topical application of L-arginine in situ in basilar arteries were observed in animals of 12-week disease duration. Currently, there is no information whether duration of disease alters arginine transport kinetics.

Transport kinetics into vascular smooth muscle cells during topical application of arginine in diabetic arteries is another consideration. This initial uptake must be sufficient to optimize intracellular arginine at a concentration adequate to facilitate subsequent uptake by diabetic endothelium. It was presumed that the concentration of arginine that failed to augment relaxation in diabetic rat basilar artery and hamster cheek pouch microcirculation was adequate on the basis of improved relaxation in basilar artery of hypertensive rats and in cheek pouch of cardiomyopathic hamsters, using a similar arginine concentration. It is possible that the concentration and time of exposure and alterations in transport into vascular smooth muscle are complications that need to be considered for diabetic tissue. Indeed, in studies conducted within the same species, the $K_m$ for transport into smooth muscle cells is much lower (ie, 25 μmol/L) compared with that for endothelial cells (ie, ≈100 μmol/L). Furthermore, the maximum capacity for arginine transport is much lower for smooth muscle compared with endothelial cells. These effects might limit the amount of arginine transported by smooth muscle cells and subsequently delivered to endothelial cells to improve NO synthesis in topical application protocols.

Alternatively, protein kinase C (PKC) is known to be increased in diabetic rat aorta and heart and in vascular smooth muscle cells exposed to elevated glucose concentrations. Furthermore, PKC inhibitors normalize relaxation in the cerebral arteries of diabetic or hyperglycemic rats. PKC activation is known to markedly reduce arginine transport into normal rat vascular smooth muscle cells. Thus, these alternative hypotheses should be addressed before final conclusions can be drawn regarding regional differences in efficacy of arginine to restore endothelial function.

The efficacy of arginine in diabetic kidney seems less in doubt. The hyperfiltration and proteinuria produced in short-term diabetes in rats was prevented by dietary arginine supplementation. Paradoxically, this was associated with a reversal of the enhanced urinary excretion of nitrate/nitrite and cGMP, suggesting that dietary arginine represses the augmented renal production of NO at early stages of the disease. In 2-month diabetic rats, arginine modestly improved the decreased basal and agonist-stimulated cGMP in glomeruli but not to control levels (cGMP). Interestingly, with slightly longer disease duration, arginine failed to alter the decreased basal and agonist-stimulated NO as measured by the NO electrode, which is in agreement with temporal studies in diabetic rat aorta.

**Conclusion.** There is increasing evidence both in vivo and in vitro that arginine can improve endothelial dysfunction and NO production in diabetes. Because of certain methodological considerations, additional studies are warranted to determine whether there are important regional differences in vascular response to arginine and whether arginine will be efficacious at various stages of the disease.

**Effects of Arginine on NO in Human Diabetes**

The possibility that NO deficits occur in clinical diabetes arises from a report showing that arginine increases plasma nitrate/nitrite levels in diabetic patients. Unfortunately, the effects of arginine in control patients was not evaluated. In one study, arginine increased nitrates and exhaled NO by nearly equivalent degrees in control and IDDM patients. The effects of arginine administration in vivo must be interpreted with caution because arginine also increases insulin secretion and the arginine stimulus might be altered by disease. Insulin is reported to cause dilation via stimulation of NO production, although one recent report shows that insulin can produce dilation in the presence of an NOS inhibitor or can be blocked by adenosine receptor antagonists or $K_{ATP}$-sensitive channel blockers.

To circumvent this limitation, one study in patients with uncomplicated IDDM revealed that arginine treatment augmented plasma cGMP levels and L-citrulline levels to a similar extent in both control and IDDM patients. This is consistent with data showing that arginine supplementation improves endothelium-dependent relaxation in NIDDM. In contrast, high concentrations of L-arginine had no effect in IDDM patients. Arginine has also been shown in NIDDM patients but not healthy controls to enhance insulin-mediated dilation.

**Conclusion.** While positive results indicate that arginine may reverse NO deficits in humans, additional studies are required to confirm or refute these initial findings.

**Are Effects of Arginine NO/cGMP–Dependent?**

It has been widely assumed that the L-arginine–induced improvement in endothelium-dependent relaxation in various other models of vascular disease reflects enhanced NO/cGMP production. The fallacy of this assumption remains to be rigorously tested in diabetic models, since NO can elicit cGMP-independent effects in some tissue. For example, L-arginine augmented endothelium-dependent relaxation in hypertensive arteries via an NO-independent pathway involving cyclooxygenase-derived reactive oxygen. Furthermore, there are studies that show that arginine analogues which inhibit NOS also alter prostanooid synthesis and $K_{ATP}$-mediated relaxation. Thus, it is possible that arginine supplementation might improve relaxation in diabetic arteries via alternative NO-independent pathways.
In diabetic rat aorta, the improvement in relaxation to acetylcholine by L-arginine could be elicited despite coadministration of either TEA, indomethacin, or catalase. This suggests that enhanced K⁺ channel activity, vasodilator prostanoids, and H₂O₂ cannot account for the beneficial effects of L-arginine. More recent studies using glibenclamide suggest that L-arginine also does not improve relaxation via enhanced K<sub>ATP</sub>-mediated relaxation (G.M.P., unpublished observations, 1997). It is not possible to conclude that similar results will be obtained in other arteries and species, since a comprehensive analysis has not been forthcoming. We do note that the beneficial effects of L-arginine in diabetic canine coronaries occurred despite the fact that all animals were treated with aspirin to inhibit cyclooxygenase activity.

In diabetic canine coronary arteries and in diabetic rat aorta, restoration of endothelial function by L-arginine cannot be accounted for by altered prostacyclin production or enhanced K⁺ channel activity. Combined with the data showing augmented cGMP generation, this suggests that the effects of arginine in diabetes are specific for enhanced NO activity.

**Effects of Arginine on Endothelial Dysfunction Produced by Elevated Glucose Concentration In Vitro or Hyperglycemia In Vivo**

One study noted that flow reduction in normal isolated guinea pig hearts resulted in vasoconstriction that was reversed by L-arginine. In contrast, the acute vasodilation produced by flow reduction in the presence of 44 mmol/L D-glucose was augmented by L-arginine. This suggests that arginine may play an important role in autoregulation under high glucose conditions.

In another study, incubation of normal rat mesenteric arteries with elevated glucose concentrations in vitro caused impaired relaxation to acetylcholine. Addition of 0.1 mmol/L L-arginine abrogated the effect of 20 mmol/L glucose and significantly reduced the detrimental effects of 45 mmol/L glucose. Another study showed that glucose-induced decrease in Na⁺,K⁺-ATPase of normal rabbit aorta was also prevented by coincubation with L-arginine.

The direct effects of glucose have also been examined in hyperglycemic clamp experiments in leg blood flow of healthy subjects. The decreased blood flow observed after 60 and 90 minutes of hyperglycemia was completely reversed by L-arginine. The fact that neither D-arginine or L-lysine was effective illustrates the selectivity and specificity for the L-arginine action.

**Conclusion.** Arginine appears to provide protection from alterations in vascular function caused by elevated glucose concentration. The mechanism of this protection is still unknown.

**Potential Actions of Chronic Arginine Treatment on Endothelial Function**

In addition to potential action on insulin release, chronic arginine administration may provide multiple benefits to ameliorate diabetes-induced endothelial dysfunction for other reasons. Arginine administration reduces lipid peroxide levels in diabetic patients. Arginine inhibits lipid peroxidation in vitro and has been reported to be a direct scavenger of superoxide anions. Other studies do not support a direct scavenger action for arginine. Alternatively, L-arginine might enhance relaxation by increasing NO release, which in turn scavenges enhanced rates of superoxide anion production that are elevated in diabetic arteries.


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Arginine and Diabetic Vasculature


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Review of Alterations in EndothelialNitric Oxide Production in Diabetes: Protective Role ofArginine on Endothelial Dysfunction
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