Review

Nitric oxide and vascular responses in Type I diabetes

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Abstract

Vascular complications are major causes of morbidity and mortality in patients with diabetes mellitus. The mechanisms underlying the development of microvascular and macrovascular angiopathy in Type I (insulin-dependent) diabetes mellitus are complex and incompletely understood. The discovery of endothelium-derived nitric oxide has greatly improved our understanding of vascular biology. Nitric oxide has an important role in the regulation of vascular tone and impaired nitric oxide activity could be implicated in the development of diabetic vasculopathy. Vascular studies of endothelial function in Type I diabetes have produced conflicting results. The role of nitric oxide in diabetic vasculopathy is still not clear. [Diabetologia (2000) 43: 137–147]

Keywords Type I diabetes, nitric oxide, endothelium, vasodilatation, plethysmography, flow-mediated dilatation, vascular ultrasound.

Diabetic microangiopathy and macroangiopathy are the principal causes of morbidity and mortality in patients with diabetes mellitus [1–3]. Patients with Type I (insulin-dependent) diabetes mellitus have a three to sixfold increased risk of cardiovascular death before the age of 60 compared with non-diabetic subjects [4]. Established risk factors for coronary heart disease (CHD) do not, however, fully explain the increased risk in Type I diabetic patients [5].

The vascular endothelium has a key role in maintaining homeostasis of the vasculature through the synthesis of vasoactive substances that modulate vascular tone, inhibit platelet aggregation and vascular smooth muscle cell (VSMC) proliferation [6, 7]. Endothelial dysfunction has been suggested to be an early event in diabetic vascular disease [8, 9]. The discovery of endothelium-derived nitric oxide (NO) in the late 1980s [10] has considerably improved our understanding in vascular biology and pathogenesis of endothelial dysfunction. With the use of techniques such as brachial artery ultrasonography, venous occlusion plethysmography and brachial artery infusion of endothelium-dependent vasoactive agents, endothelial function can be indirectly assessed in different vascular beds. Most published reviews have focused on Type II (non-insulin-dependent) diabetes mellitus rather than Type I diabetes. We reviewed the current evidence for endothelium-mediated vascular dysfunction in Type I diabetes following an extensive literature search from both Medline and PubMed (1965–1999) using the following keywords: 'Type I diabetes mellitus', 'endothelial function', 'nitric oxide', 'vasodilatation'. Potential mechanisms which could be involved in endothelial dysfunction in Type I diabetes are discussed.

Corresponding author: Dr. N. N. Chan, EURODIAB, Department of Epidemiology & Public-Health, 1–19 Torrington Place, University College London, London WC1E 6BT, United Kingdom
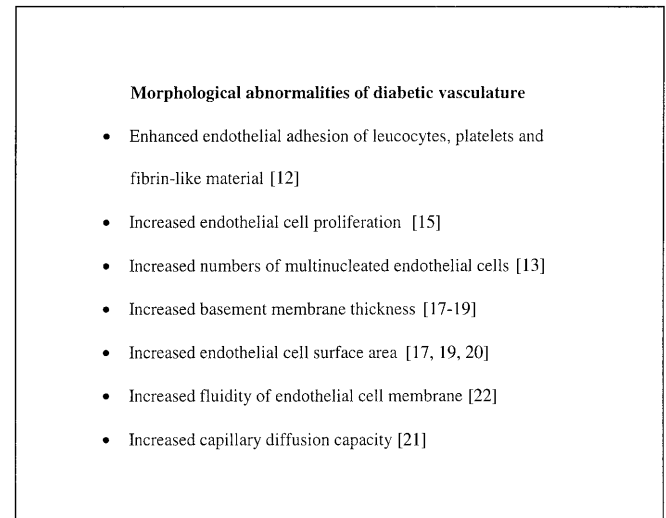*Abbreviations*: CHD, Coronary heart disease; NO, nitric oxide; STZ, streptozotocin; BM, basement membrane; VSMC, vascular smooth muscle cell; cGMP, cyclic guanosine monophosphate; L-NMMA, N^G-monomethyl-L-arginine; NOS, nitric oxide synthase; SNP, sodium nitroprusside; FMD, flow-mediated dilatation; PKC, protein kinase C; DAG, diacylglycerol; AGE, advanced glycation end product.

Morphology of vasculature in diabetes

The vascular endothelium forms the lining of the blood vessel wall separating the lumen from the vascular smooth muscle. Under the electron microscope, normal endothelial cells have a cobble-stone appearance with gap junction formation in between cells. A basement membrane separates this single layer of endothelial cells from the smooth muscle. Changes in morphology of the vasculature in diabetes have been characterised in both animal and human models (Fig. 1). Rats are the animal model commonly used and are rendered diabetic by treatment with streptozotocin (STZ), a nitrosamine which is toxic to the pancreatic beta-cell. In other species such as the rabbits which are more resistant to STZ [11], alloxan is used to induce diabetes.

In alloxan-induced diabetic rabbits, endothelial alterations occur in the aorta as early as 2 weeks after onset of diabetes and the changes become more severe by 6 weeks [12]. These alterations are consistent with injury and include adhesion of leucocytes, platelets and fibrin-like material to the endothelial surface [12]. Using cultured aortic endothelial cells from STZ diabetic minipigs, endothelial cells derived from diabetic minipigs have been shown to have a higher rate of proliferation and a higher percentage of large multinucleated cells [13]. Furthermore, these multinucleated cells have an increased low-density lipoprotein binding and degradation compared with non-diabetic controls [13]. In STZ-induced diabetic rats, an increased rate of aortic endothelial cell death as well as an increase in endothelial permeability in the aorta was observed at 6 weeks after onset of diabetes [14].

In humans, an increase in basement membrane (BM) thickness of the microvasculature is the major feature in Type I diabetes. Using transmission electron microscopy, BM thickening has been shown in skin capillaries in patients with Type I diabetes compared with non-diabetic subjects [15]. Additionally, it has been shown that one year of intensive glycaemic control in Type I diabetes reduces BM width in skeletal muscle capillaries [16]. Furthermore, diabetic neuropathy has been shown to be associated with an increase in BM thickening [17–19], numbers of endothelial nuclei [17], endothelial cell area [17,19,20] and capillary diffusion capacity as measured by clearance of radiolabelled xenon and iodide from hyperaemic leg muscles [21]. Recently, more features of endothelial alteration in diabetes were shown by transmission electron microscopy and fluorescence anisotropy using specific fluorescent probe anchoring at the endothelial surface membrane [22]. These included an increase in fluidity of the endothelial membrane, an increase in mitochondrial area and a more fluid phase endocytosis in endothelial cells obtained from umbilical cords of Type I diabetic pregnant women compared with non-diabetic control subjects [22].

Morphological abnormalities of diabetic vasculature

- Enhanced endothelial adhesion of leucocytes, platelets and fibrin-like material [12]
- Increased endothelial cell proliferation [15]
- Increased numbers of multinucleated endothelial cells [13]
- Increased basement membrane thickness [17-19]
- Increased endothelial cell surface area [17, 19, 20]
- Increased fluidity of endothelial cell membrane [22]
- Increased capillary diffusion capacity [21]

Fig. 1. Morphological abnormalities of diabetic vasculature

The functional relevance of these structural abnormalities is not clear as yet but they could contribute to vasculopathy and premature atherosclerosis in Type I diabetes.

L-arginine: nitric oxide: cGMP pathway

In 1980, endothelium-dependent relaxation was shown in vascular tissues [23]. This was found to be mediated by endothelial-derived relaxing factor, which has subsequently been identified as nitric oxide (NO) [10,24]. Endothelial-derived NO is synthesised from the guanidine-nitrogen terminal of the amino acid L-arginine by the endothelium isoform of NO synthase (eNOS) [25,26]. Nitric oxide has a half-life of only a few seconds [27] and is rapidly oxidised to nitrate by oxygenated haemoglobin, molecular oxygen and superoxide anions, before being excreted into the urine [28]. In addition to being a very potent vasodilator, endothelium-derived NO also has anti-atherogenic properties including decreasing platelet and leucocyte adhesion to the endothelium and inhibition of VSMC migration [6]. These effects are mediated largely through activation of guanylate cyclase, leading to increases in cyclic guanosine monophosphate (cGMP) within platelets or smooth muscle cells (Fig. 2) [29, 30].

Nitric oxide is released under physiological conditions [31] regulating basal blood flow in healthy humans [32]. This basal NO release is inhibited by N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of NOS [32]. Endothelial NO synthesis can also be stimulated by a variety of physical and biochemical stimuli. These include shear stress [33, 34], pulsatile stretching of the vessel wall [35, 36], low arterial oxygen tension [37], receptor-dependent agonists (e.g. acetylcholine, bradykinin) and receptor-inde-
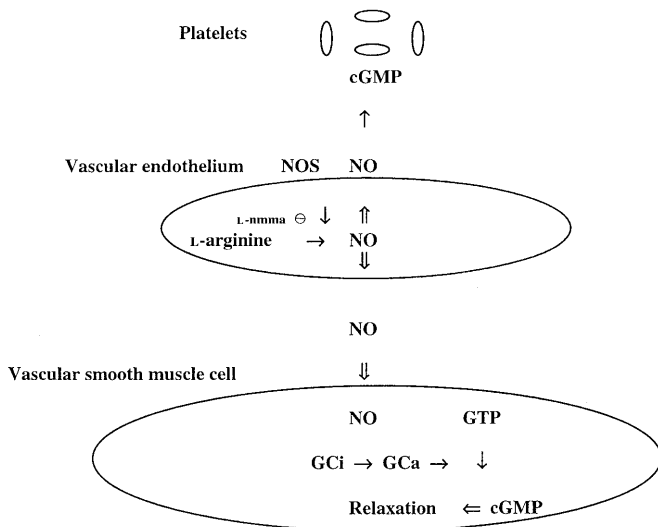
Fig. 2. The nitric oxide: L-arginine pathway. Endothelial-derived nitric oxide (NO) is synthesised from L-arginine by NO synthase (NOS). Nitric oxide diffuses across to vascular smooth muscle cell and platelets to cause vasorelaxation and inhibition of platelet aggregation, respectively. Both processes are mediated through cyclic GMP (cGMP). Synthesis of NO can be inhibited by L-N^G-monomethyl-arginine (L-NMMA), an NOS-inhibitor. GCi, inactive guanylate cyclase; GCa, active guanylate cyclase

pendent agonists (e.g. calcium ionophores, polycations or calcium-ATPase inhibitors) [10, 38, 39]. Determination of vasoconstriction in response to L-NMMA or vasodilatation in response to acetylcholine in a vascular bed or an isolated blood vessel has been used to infer basal NO release and stimulated NO-mediated vasodilatation respectively, in human and animal models with diabetic vascular disease [40].

Defective response to NO or reduced NO availability in Type I diabetes?

Given the potential antiatherogenic properties of NO, it is possible that a defect in the L-arginine: NO: cGMP pathway contributes to the increased cardiovascular risk in Type I diabetes. This defect could occur at one or several stages of the pathway. There could be reduced basal or stimulated NO release or both, decreased bioavailability of NO or reduced VSMC responsiveness. Experimental evidence suggests that all these defects are plausible in Type I diabetes. For example, it has been shown that the vasodilating effect of insulin in skeletal muscle is mediated through an increase in NO release [41]. Hence in the presence of insulin deficiency in Type I diabetes, there could be reduced NO release. Even if NO release is normal, its bioavailability could be reduced because advanced glycation end products, as a result of chronic hyperglycaemia, quench NO in vitro [42]. Alternatively, there could be defective VSMC re-

sponse to NO as hyperglycaemia interferes with NO-induced guanylate cyclase activation [43].

Endothelium-dependent vascular responses in Type I diabetes

As discussed below [44, 45] studies assessing endothelial function in both animal and human models of Type I diabetes have produced conflicting results. In experimental models, endothelial function is modulated by a number of factors associated with Type I diabetes including degree of acute hyperglycaemia [46–49], diabetes duration (possibly associated with accumulation of advanced glycation end products) [42, 50], insulin concentrations [51, 52], and the presence or absence of diabetic complications such as autonomic neuropathy [53] and microalbuminuria [54]. Variation in these factors between different studies might account for the conflicting results.

Animal studies

In conduit vessel studies, endothelium-dependent relaxation in diabetic animal models has been reported to be either impaired [55, 56] or normal [57–60] compared with controls. A similar disparity has also been observed in resistance vessels with endothelium-dependent relaxation being found to be impaired [61–64] or normal [65]. In some animal studies, endothelium-dependent relaxation has even been enhanced [66, 67]. This disparity is possibly due to differences in disease duration, the vascular bed studied and methods of vessel preparation (e.g. using helical strips [68]) which determine the extent of vascular endothelium preservation. For example, there is a substantial difference in the timing between impairment of endothelial-dependent relaxation and onset of diabetes in different vascular beds, ranging from 1 week in intestinal arterioles [69] to between 4 and 6 weeks in mesenteric arteries [70, 71]. An additional factor to consider is the possible cross-over effects due to multiple drug application in the same specimen [57].

The importance of disease duration has been highlighted in a recent study [72]. Using aortic rings of STZ-induced diabetic rats, pre-contracted with norepinephrine, endothelium-dependent relaxation to acetylcholine was increased at 24 h after injection with STZ, normal after 1 and 2 weeks of disease and impaired at 8 weeks of disease compared with controls [72]. In both control and diabetic aortic rings, acetylcholine-induced relaxation was blocked using L-nitroarginine suggesting that the enhanced response was mediated through NO [72]. This is the first study to show the triphasic response in relation to disease duration in diabetic animal models.

Table 1. Summary of in vivo endothelial function studies by venous occlusion plethysmography in human Type I diabetes mellitus

Authors	Numbers (female/male)	Mean disease duration (years)	HbA$_{1c}$ (%)	Euglycaemic-insulin clamp	Diabetic complications	Stimulated NO response	Endothelium-independent response
Venous occlusion plethysmography							
Calver et al [82]	10 (0/10)	"Recent onset"	6.7 ± 0.5	No	without	unchanged	unchanged (verapamil) impaired (SNP)
Johnstone et al [76]	15 (11/4)	14 ± 2	11.9 ± 0.6	No	not stated	impaired	unchanged (SNP + verapamil)
O'Driscoll et al [78]	9 (0/9)	18 ± 2	8.3 ± 0.4	Yes	without	impaired	unchanged (SNP)
Huvers et al [80]	34 (7/27)	17	8.98	Yes	with and without	unchanged	unchanged (SNP)
Elliott et al [54]	14 (6/8)	20.7	3.3[a]	Yes	normoalbuminuria	impaired	unchanged (SNP)
	14 (3/11)	22.6	3.3[a]	Yes	microalbuminuria	unchanged	unchanged (SNP)
Halkin et al [81]	18 (2/16)	12.0 ± 8.0	4.074 ± 0.207[a]	No	without	unchanged	unchanged (SNP)
Smits et al [79]	11 (0/11)	15.1 ± 8.2	9.2 ± 0.9	No	without	unchanged	unchanged (SNP)
Makimattila et al [53]	10 (0/10)	28 ± 3	8.6 ± 0.3	Yes	macroalbuminuria & autonomic dysf.	enhanced	enhanced (SNP)
	12 (0/12)	18 ± 3	8.6 ± 0.3	Yes	microalbuminuria & autonomic dysf.	unchanged	unchanged (SNP)

[a] Fructosamine (mmol/l)

Human studies

In vitro studies

There have been two studies on in vitro endothelium-dependent relaxation. Using isolated resistance arteries (from biopsy specimens of subcutaneous fat from the gluteal region) from Type I diabetic patients, impaired relaxation to acetylcholine but not to bradykinin or sodium nitroprusside (SNP) has been shown in pre-contracted small arteries [73]. The normal vascular response to bradykinin in that study suggests defective endothelial cell acetylcholine receptor excitation-coupling in Type I diabetes rather than a reduction in NO synthesis [73]. Recently, resistance vessels dissected from gluteal fat biopsy specimens in normotensive Type I diabetic patients with varying degrees of microvascular complications were examined. The preliminary data showed no difference in acetylcholine-induced relaxation compared with non-diabetic specimens [74].

In vivo studies

1. Forearm venous occlusion plethysmography

Agonist-stimulated vascular responses. This technique has been used to study human resistance arteries [75] in Type I diabetes. Endothelium-dependent vasodilatation is assessed by intra-arterial (brachial artery) infusion of muscarinic agonists (acetylcholine, methacholine or carbachol). Using this method, endothelium-dependent relaxation in Type I diabetic patients has been shown to be impaired [76–78], normal [54, 79–81] and even enhanced [53] (Table 1).

Vasodilator response to nitric oxide donors. Endothelium-independent vasodilatation in Type I diabetic patients has been assessed extensively by intra-arterial infusion of sodium nitroprusside, an NO donor. Most studies using venous occlusion plethymography found that vascular response to SNP was not changed. In one study, vascular response to SNP was found to be inversely correlated with Na$^+$/Li$^+$ counter-transport [81], a possible marker for the development of diabetic vascular complications. In another study a diminished response to SNP was, however, observed [82] which could be attributable to reduced VSMC sensitivity to NO. Verapamil was used in two studies as an NO-independent vasodilator [76, 82] to determine whether any impaired vasodilatation to SNP is specific for NO pathways. In both studies, vascular response to verapamil was unchanged.

Vasoconstrictor response to nitric oxide synthase inhibitor. In the assessment of basal NO release using intra-arterial infusion of L-NMMA, data are also inconsistent. The vasoconstrictor response to L-NMMA was unchanged in one study [80] but blunted in two other studies [54, 82]. This blunted response was found to be most pronounced in Type I diabetic patients with microalbuminuria [54].

Table 2. Summary of in vivo endothelial function studies by vascular ultrasound in human Type I diabetes mellitus

Authors	Numbers (W/M)	Mean disease duration (years)	HbA$_{1c}$ (%)	Euglycaemic-insulin clamp	Diabetic complications	Reactive hyperaemia	Endothelium-inde-pendent response
Flow-mediated vasodilatation assessed by high resolution vascular ultrasound							
Clarkson et al [92]	80 (40/40)	13 ± 8	9.5 ± 2.2	No	with and without	impaired	impaired (GTN)
Zenere et al [85][a]	10 (5/5)	10 ± 1	7.7 ± 0.2	No	normoalbuminuria	impaired	impaired (GTN)
	8 (6/2)	11 ± 1	7.2 ± 0.5	No	microalbuminuria	impaired	impaired (GTN)
Lambert et al [84]	52 (22/30)	14.9 ± 8	7.9 ± 1.2	No	retinopathy only	unchanged	unchanged (GTN)
Enderle et al [86]	17 (10/7)	21.5 ± 10.2	8.0 ± 1.1	No	without	unchanged	unchanged (GTN)
Lekakis et al [87]	5 (4/1)	20 ± 8.5	7.1 ± 1.0	No	microalbuminuria	impaired	impaired (ISDN)
	26 (17/9)	12.9 ± 8.4	6.5 ± 1.5	No	normoalbuminuria	impaired	unchanged (ISDN)
Mecking et al [93]	18 (10/8)	27.8 ± 2.4	10.5 ± 2	No	microalbuminuria	impaired	unchanged (GTN)
	18 (10/8)	26.9 ± 2.0	9.6 ± 0.3	No	normoalbuminuria	impaired	unchanged (GTN)

[a] The common femoral artery was studied by echo-ultrasound, W/M = women/men

2. Flow-mediated dilatation assessed by vascular ultrasound

Endothelial function of conduit vessels (mainly the brachial artery) has been evaluated using high resolution vascular Doppler ultrasound [83] to determine the degree of flow-mediated dilatation (FMD) [84–87]. The brachial artery is used because it is easily accessible and there is some evidence that endothelial dysfunction in brachial artery parallels that of the coronary artery [88]. Although this method of evaluating endothelial function is non-invasive, it has the disadvantage that a highly skilled ultrasonographer is required for imaging to be done accurately and reproducibly. This technique has a relatively low within person reproducibility [89]. Furthermore, differences in the flow velocity profile during reactive hyperaemia could lead to poor reproducibility of flow-mediated dilatation [90]. The brachial arteries have mostly been used although one study assessed the common femoral artery [85]. The results are again conflicting with endothelium-dependent vascular responses shown to be impaired [85, 87, 91] or unchanged [84, 86] (Table 2).

Similarly, endothelium-independent vascular response to glyceryl trinitrate was found to be either impaired [85, 90] or unchanged [86, 91]. In studies showing reduction in FMD in Type I diabetic patients, there does not seem to be a relation with presence or absence of microalbuminuria [90, 92].

Conflicting data in human in vivo studies: potential factors

Sex difference. The sex difference in CHD incidence is abolished in Type I diabetes [4]. If there is any differential effect of diabetes on endothelial function between the sexes, then differences between studies could arise if the proportion of women in the studies differs. Furthermore, forearm length (and hence vessel length) is different between men and women. This could result in differences in vascular responses to acetylcholine [93] because acetylcholine is rapidly destroyed by cholinesterase enzymes and the magnitude of response is partially dependent on forearm length [94]. With the exception of one study [76], venous occlusion plethysmography studies have been done predominantly in male diabetic subjects [54, 80] with female diabetic subjects not being included at all in some studies [53, 78, 79, 82]. There is some suggestion from table 1 that many of the negative studies are those which have included relatively few women. Hence, it is possible that the different male to female ratio between studies contributed to the conflicting results.

Effect of glycaemic control. There is a large variation in mean HbA$_{1c}$, or glycated haemoglobin, in Type I diabetic patients included in different studies. The greatest contrast is between HbA$_{1c}$ 6.7% [82] and HbA$_{1c}$ 11.9% [76]. In the first study stimulated endothelium-dependent vasodilatation was not changed and in the second it was impaired. Most research groups have studied patients with suboptimal glycaemic control with HbA$_{1c}$ ranging from 8.3–9.2% [53, 78–80].

Degree of acute hyperglycaemia. There is substantial evidence that acute hyperglycaemia attenuates endothelium-dependent vasodilatation [47, 48]. It is possible that the variable degree of hyperglycaemia at the time of measurement in different studies had an effect on endothelium-dependent vascular responses. To minimise the acute hyperglycaemic effect during studies, some groups have used the euglycaemic-insulin clamp method to maintain normoglycaemia [78, 80].

Different muscarinic agonists. Methacholine, acetylcholine and carbachol have all been used to stimulate NO production. Direct comparisons between studies

using different muscarinic agonists should be made with caution. It has been shown in some studies that vasodilatation response to acetylcholine is attenuated by L-NMMA whereas that to methacholine is not [95].

Effects of diabetic complications. Several groups have used Type I diabetic patients without microvascular complications in whom endothelium-dependent responses were found either not to be changed [79, 82] or to be impaired [54, 78]. In the only human in vivo study with Type I diabetic patients where autonomic dysfunction was documented, hyperresponsiveness to acetylcholine (as well as to SNP, an endothelium-independent vasodilator) was found in those with macroalbuminuria [53]. It has been suggested that this hyperresponsiveness to acetylcholine is a result of increased sensitivity of the VSMC as it would also explain the hyperresponsiveness to SNP [96].

An important limitation in all these in vivo human studies using forearm venous occlusion plethysmography is that although patients with clinical macrovascular complications are excluded, it is possible that some patients have subclinical macrovascular complications (asymoptomatic atherosclerosis) which has a relevant effect on endothelial function. It has been shown in a recent study using electron beam computed tomography that there is a high prevalance of coronary artery calcification (a validated measure of atheroma burden) in young (age 30–55) patients with Type I diabetes [97]. Furthermore, the studies have all been quite small and do not allow evaluation of the effects of concomitant diabetic complications. To establish the important determinants of endothelial function in Type I diabetes, a large study is needed in which the effect of factors such as sex, disease duration, diabetic complications and glycaemic control can be evaluated.

Potential mechanisms for endothelial dysfunction in Type I diabetes

The mechanisms whereby Type I diabetes is associated with endothelial dysfunction are complex and not completely understood. Although the concomitant presence of hypertension and dyslipidaemia in Type I diabetes could contribute directly to endothelial dysfunction, a combination of several mechanisms directly related to increased glucose concentration could also be responsible and are the focus of the remainder of this paper.

Role of free radicals

There is substantial evidence to indicate that hyperglycaemia-induced endothelial dysfunction is mediated by free radicals produced through increased arachidonic acid metabolism [98–100]. It has been shown that in human aortic endothelial cells, prolonged exposure to high concentrations of glucose increases *eNOS* gene expression and NO release. This is associated, however, with a pronounced concomitant increase of superoxide anion production [101]. These anions inactivate NO and furthermore, they interact with NO leading to production of peroxynitrite [102, 103], a potent oxidant, which in turn stimulates cyclooxygenase catalysis, lipid peroxidation and increased prostanoid production resulting in endothelial dysfunction [104, 105]. Superoxide dismutase, a scavenger of superoxide anion, has been shown to normalise NO-mediated vasorelaxation impaired by increased glucose concentrations [98, 106, 107]. Recently, NO synthesis and oxidative stress have been quantified by measuring radiolabelled nitrite and 8-epiPGF2α [108]. The preliminary results showed an inverse relation between NO synthesis and free radical activity [108]. Thus hyperglycaemia could induce increased NO production as well as reduced NO availability due to inactivation mediated by free radicals.

Aldose reductase and the polyol pathway

Chronic hyperglycaemia increases aldose reductase activity leading to an increase in glucose metabolism through the polyol pathway [109, 110] in which it is first metabolised to sorbitol and subsequently to fructose (Fig. 3). Aldose reductase has been shown to be present in the vascular endothelial cells [111] and is the rate-limiting enzyme in the initial conversion of glucose to sorbitol. This process is dependent on nicotinamide-adenine dinucleotide phosphate (NADPH) and therefore results in an increase in its use [109, 112]. Because NADPH is also an essential cofactor for NOS for the synthesis of NO, its depletion as a result of chronic hyperglycaemia could lead to a reduction in NO production. Aldose reductase inhibitors have shown early promise in reversing glucose-induced changes in sorbitol and *myo*-inositol metabolism and endothelial dysfunction in experimental diabetes [113–115]. It remains to be determined, however, whether aldose reductase inhibitors have a part in the prevention of atherosclerosis in humans.

Protein kinase C

Activation of protein kinase C (PKC) by increases in diacylglycerol (DAG) induced by hyperglycaemia has been suggested as a mechanism for endothelial dysfunction and vascular complication in diabetes [116, 117]. Such activation has been shown to be associated with increased urinary albumin excretion in rats [117]. Indeed, PKC activation is responsible for

Glucose

| Aldose reductase | ⬇ | ⟶ NADPH |

Sorbitol ⟶ NADP⁺

| Sorbitol dehydrogenase | ⬇ | ⟶ NAD⁺ |
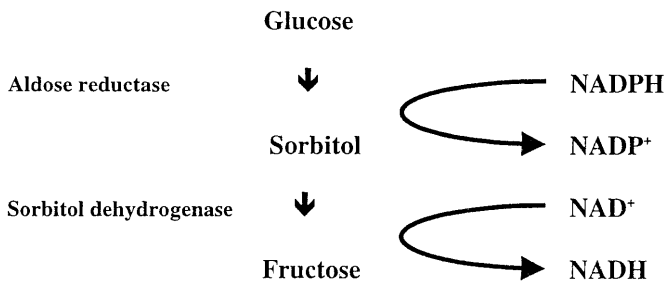
Fructose ⟶ NADH

Fig. 3. The polyol pathway: glucose is converted by aldose reductase to sorbitol which is metabolised to fructose by sorbitol dehydrogenase. This process is dependent on nicotinamide-adenine dinucleotide phosphate (NADPH) and oxidised nicotinamide-adenine dinucleotide (NAD^+)

several vascular alterations in diabetes such as a decrease in the activity of Na^+-K^+-adenosine triphosphatase, increases in extracellular matrix, cytokines, permeability, contractility and cell proliferation [116] and an increase in vasoconstrictor prostanoids [118]. Isolated rabbit aorta exposed to increased glucose concentrations had impaired endothelium-dependent relaxation to acetylcholine after 10 min treatment with 4-phorbol 12-myristate 13-acetate, a PKC activator [118]. Indomethacin increased relaxation induced by acetylcholine, suggesting a role for vasoconstrictor prostanoids, and this abnormal relaxation was restored with sphingosine, a PKC inhibitor [118].

Advanced glycation end products

In the presence of sustained high plasma glucose concentrations, circulating or tissue-structure proteins including arterial wall collagen and glomerular basement-membrane proteins undergo covalent, non-enzymatic glycation and cross-linking resulting in the formation of advanced glycation end products (AGEs) [119, 120]. Advanced glycation end products possibly contribute to endothelial dysfunction and diabetic vascular complication by permanent chemical modification of circulating cells and proteins, and indirectly by stimulating cellular response through receptors specific for AGE-modified proteins [121, 122]. These specific receptors for AGE-modified proteins have been identified in murine and human macrophage cells which are thought to have a key role in the clearance of AGE-modified proteins [121, 122]. Rate of accumulation of AGEs seems to be faster than normal in arteries and circulation of patients with diabetic nephropathy consistent with the renal tract being the main, if not only, source of clearance [123]. Accumulation of AGEs over time has been shown to reduce NO availability. Studies using a rat model of STZ-induced diabetes showed both in vitro and in vivo that early in the advanced glycation pathway, reactive intermediates form which then react

with and quench NO rapidly (< 5 s) as a result of direct reaction between NO radical and the AGEs [124]. In addition to NO inactivation, AGEs have been shown to impair the effects of NO on mesangial cells antiproliferation, an early and characteristic lesion of diabetic vasculopathy [125].

Aminoguanidine, an inhibitor of AGEs formation [126], has been shown in diabetic rats to partially restore endothelium-dependent relaxation in vivo [124] and to reduce albuminuria associated with hypertension [126] and to retard the development of diabetic nephropathy [127]. It should, however, be noted that aminoguanidine has multiple actions including direct effects on NO generation.

Conclusions

Studies in experimental animals and in vitro have shown that loss of NO is associated with increased vascular reactivity to constrictors, enhanced platelet adhesion and aggregation, increased adhesion of leucocytes, promotion of VSMC growth and accelerated atherogenesis [40]. Type I diabetes could affect NO production through generalised actions on the endothelium or through specific effects on the NO pathway. For example, there could be specific defects in signal transduction mechanisms linked to NO synthase (receptors, ion channels), NO synthase expression, post-translational modification of the enzyme or destruction of the NO once it has been made. In addition, it is possible that down-regulation of guanylate cyclase or the other effector mechanisms of NO could be affected. Although many studies in animal models have indicated that Type I diabetes is indeed associated with functional defects in parts of the NO pathway, in humans the data are conflicting and there is no clear consensus about the level at which the disease might alter NO signalling. The variable results of endothelial function obtained in different studies may be partially explained by differences in methodology, blood vessel size and the presence of diabetic complications. The mechanisms underlying endothelial dysfunction could involve several biochemical pathways with an increase in glucose concentration providing the initial metabolic insult. One area which has not been studied in depth is the effect of abnormal neural control on endothelial function. Future arterial forearm studies with occlusion venous plethysmography should include sufficient numbers of patients to assess the role of autonomic dysfunction on vascular reactivity.

At present, several compounds have received attention in the prevention of diabetic vascular dysfunction and complications, including antioxidants such as vitamin C, aldose reductase inhibitors, PKC inhibitors, aminoguanidine, L-arginine all of which have shown early promise in animal studies. The effi-

cacy of these agents in endothelial function in humans has, however, yet to be shown. Beyond that it is then important to establish whether improvement in endothelial function can be translated into a reduction in mortality and morbidity.

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