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REVIEW

Endothelial dysfunction in diabetes

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Endothelial dysfunction plays a key role in the pathogenesis of diabetic vascular disease. The endothelium controls the tone of the underlying vascular smooth muscle through the production of vasodilator mediators. The endothelium-derived relaxing factors (EDRF) comprise nitric oxide (NO), prostacyclin, and a still elusive endothelium-derived hyperpolarizing factor (EDHF). Impaired endothelium-dependent vasodilation has been demonstrated in various vascular beds of different animal models of diabetes and in humans with type 1 and 2 diabetes. Several mechanisms of endothelial dysfunction have been reported, including impaired signal transduction or substrate availibility, impaired release of EDRF, increased destruction of EDRF, enhanced release of endothelium-derived constricting factors and decreased sensitivity of the vascular smooth muscle to EDRF. The principal mediators of hyperglycaemia-induced endothelial dysfunction may be activation of protein kinase C, increased activity of the polyol pathway, non-enzymatic glycation and oxidative stress. Correction of these pathways, as well as administration of ACE inhibitors and folate, has been shown to improve endothelium-dependent vasodilation in diabetes. Since the mechanisms of endothelial dysfunction appear to differ according to the diabetic model and the vascular bed under study, it is important to select clinically relevant models for future research of endothelial dysfunction.
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Abbreviations: ACh, acetylcholine; AGE, advanced glycation end product; EDCF, endothelium-derived constricting factors; EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; NO, nitric oxide; SOD, superoxide dismutase; STZ, streptozotocin

Introduction

Macro- and microvascular disease are currently the principal causes of morbidity and mortality in patients with type I and type II diabetes mellitus. Loss of the modulatory role of the endothelium may be a critical and initiating factor in the development of diabetic vascular disease.

Endothelial cells actively regulate basal vascular tone and vascular reactivity in physiological and pathological conditions, by responding to mechanical forces and neurohumoral mediators with the release of a variety of relaxing and contracting factors (Furchgott & Vanhoutte, 1989). The endothelium-derived relaxing factors (EDRFs) include nitric oxide (NO), prostacyclin and an, as yet elusive, endothelium-derived hyperpolarizing factor (EDHF) (Feletou & Vanhoutte, 1999). The activity of the endothelium extends, however, far beyond the control of vascular tone and reactivity, and the release of vasodilating mediators clearly reflects only one aspect of the homeostatic and protective role of the endothelium. Nevertheless, endothelium-dependent vasodilatation is generally used as a reproducible and accessible parameter to probe endothelial function in different pathophysiological conditions.

The present communication reviews the extant experimental and clinical research on disordered endothelium-dependent vasodilatation in diabetes, with a focus on those studies ancillary to a better understanding of its mechanisms and aetiology.

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Although strict glycaemic control delays the onset and slows down the progression of diabetic vascular complications (The DCCT Research Group, 2000), this strategy is not successful in all patients. The knowledge obtained from the studies on endothelial dysfunction has given impetus to the search for novel approaches in the prevention and treatment of diabetic vascular disease. These alternative strategies will be particularly suitable for those diabetic patients that are unable to achieve a strict metabolic control and will be addressed by the current review where appropriate.

Experimental and clinical evidence for the presence of impaired endothelium-dependent vasodilatation in type I and II diabetes

Whereas some of the earlier reports showed normal (Wakabayashi *et al*., 1987; Head *et al*., 1987; Mulhern & Docherty, 1989) or even enhanced (White & Carrier, 1986; Bhardwaj & Moore, 1988; Gebremedhin *et al*., 1988) endothelium-dependent vasodilatation, impaired responses to different endothelium-dependent agonists have been repeatedly and consistently demonstrated in different vascular beds of both chemically-induced and genetic models of type I diabetes (Table 1A–C). Similarly, impaired endothelium-dependent vasodilatation has been demonstrated in patients with type I diabetes in the absence of clinical complications, although several studies failed to confirm these findings (Table 2A). The discrepancies are most likely due to differences in the clinical characteristics of the study population. Not surprisingly,

Table 1 Experimental studies of impaired endothelium-dependent vasodilatation with attempts to restore the defect.

A: Isolated vessels

Reference	*Diabetes model*	*Vessel*	*EDVD*	*EIVD*	*Restoration*
Tesfamariam *et al.* 1989	rabbit, AL, 6w	Abdominal aorta	ACh↓, A23187↔	SNP↔	COX blockade (T) TP-RA (T)
Tesfamariam *et al.*, 1990	rabbit, high glucose, 6h	Abdominal aorta	ACh↓, A23187↔	SNP↔	COX blockade (T) TP-RA (T) TXA_2-S blockade (N)
Tesfamariam *et al.*, 1991	rabbit, high glucose, 6h	Abdominal aorta	ACh↓, ADP↓, A23187↔	SNP↔	PKC blockade (T)
Tesfamariam & Cohen, 1992	rabbit, high glucose, 6h and AL, 6w	Abdominal aorta	ACh↓		SOD, catalase, allo-purinol, desferoxamine (T) probucol 6w (T)
Tesfamariam *et al.*, 1993	rabbit, AL, 6w	Abdominal aorta	ACh↓, adenosine↓	SNP↔	ARI 6w (T)
Hattori *et al.*, 1991	rat, STZ, 8-12w	Thoracic aorta	ACh↓, ADP↓, Histamine↓,	NO↓, SNP↔	SOD (T) catalase, allopurinol, desferoxamine (N) COX blockade (N)
Cameron & Cotter, 1992	rat, STZ, 3m	Thoracic aorta	ACh↓, A23187↓,	GTN↔ cromakalim↓	ARI 3m (T) COX blockade (N)
Shimizu *et al.*, 1993	rat, STZ, 10w	Thoracic aorta	ACh↓		TP-RA (T) TXA2-S blockade (N)
Otter & Chess-Williams, 1994	rat, STZ, 2w	Thoracic aorta	carbachol↓	SNP↔ forskolin↔	ARI 2w (T)
Pieper & Peltier, 1995	rat, STZ, 2m	Thoracic aorta	ACh↓	Nitroglycerin↔	L-arginine (T)
Keegan *et al.*, 1995	rat, STZ, 2m	Thoracic aorta	ACh↓	GTN↔	vitamin E 2m (P)
Pieper *et al.*, 1996	rat, BB, 2m	Thoracic aorta	ACh↓		SOD (P), COX blockade (N) aminoguanidine (N)
Pieper *et al.*, 1997	rat, STZ, 2m	Thoracic aorta	ACh↓	Nitroglycerin↔	DETAPAC, SOD + catalase (T) SOD, catalase, mannitol (N)
Pieper & Siebeneich, 1998	rat, STZ, 2m	Thoracic aorta	ACh↓	SNP↔	N-acetylcysteine (T)
Taylor *et al.*, 1992	rat, STZ, 5-6w	Mesenteric artery	ACh↓	SNP↔	COX blockade (N)
Diederich *et al.*, 1994	rat, STZ, 6-24w	Mesenteric artery	ACh↓, Histamine↓, ADP↔	SNP↑ 6w,↔12-24w verapamil↔	DTMU (T), SOD (P) COX blockade (N) TP-RA (N)
Heygate *et al.*, 1995	rat, BB, 6-8w	Mesenteric artery	ACh↓, BK↓	SNP↔	COX blockade (P) SOD, catalase, L-arginine(N) TP-RA (N)
Fukao *et al.*, 1997	rat, STZ, 8-12w	Mesenteric artery	ACh↓	Pinacidil↔	COX blockade (N) SOD (N)
Palmer *et al.*, 1998a	rat, STZ, 4-5w	Mesenteric artery	ACh↓	SNP↔	vitamin C (N) vitamin C + vitamin E (N)
Palmer *et al.*, 1998b	rat, STZ, 4-5w	Mesenteric artery	ACh↓	SNP↔	simvastatin (N) probucol (N)
Dai *et al.*, 1993	rat, STZ, 6-24w	Interlobar artery	ACh↓, Histamine↓	SNP↔6w,↓12-24w verapamil↔	DTMU (T) SOD (N)
Hill & Ege, 1994	rat, STZ, 4-6w	Skeletal muscle art.	ACh↓		aminoguanidine 4-6w (N)
Koltai *et al.*, 1997	dog, AL, 3m	Coronary artery	ACh↓	SNP↔	L-arginine (N)

B: Isolated perfused organs

Reference	*Diabetes model*	*Organ*	*EDVD*	*EIVD*	*Restoration*
Taylor *et al.*, 1994b	rat, STZ, 2-10w	Mesentery	ACh↓		ARI 2-10w (N)
Rösen *et al.*, 1996	rat, STZ, 5-26w	Heart	serotonin↓	SNP↔	vitamin E 5-26w (T) SOD (P)
Quilley *et al.*, 1996	rat, STZ, 4-6w	Heart	BK↓		ARI 4-6w (T) COX blockade (N)
Fulton *et al.*, 1996	rat, STZ, 4-6w	Kidney	ACh↓, BK↓	SNP↔	L-arginine (P) COX blockade (N)

(Continued)

Table 1 (Continued)

C: *In vivo* studies

Reference	*Diabetes model*	*Vascular bed*	*EDVD*	*EIVD*	*Restoration*
Bucala *et al*., 1991	rat, STZ, 0.5-12m	Blood pressure	ACh↓	Nitroglycerin↔	aminoguanidine (P)
Mayhan *et al*., 1991	rat, STZ, 2.5-3.5m	Pial arterioles	ACh↓, ADP↓	Nitroglycerin↔	COX blockade (T) TP-RA (T)
Mayhan & Patel, 1995	rat, high glucose 30 min	Pial arterioles	ACh↓, ADP↓, Histamine↓	Nitroglycerin↔	PKC blockade (T)
Mayhan *et al*., 1997	hamster, STZ, 2w	Cheek pouch arterioles	Substance P↓ Histamine↓	Nitroglycerin↔	L-arginine (N)
Mayhan, 1997	rat, STZ, 2-2.5m	Basilar artery	ACh↓, BK↓	Nitroglycerin↔	SOD (P)
Mayhan & Patel, 1998	rat, STZ, 3-4w	Basilar artery	ACh↓, Substance P↓	SNP↔	DMTU (T)
Bohlen & Lash, 1993	rat, high glucose, 1h	Mesenteric arterioles	ACh↓	SNP↔	SOD, catalase (T) COX blockade (P)
Pelligrino *et al*., 1994	rat, STZ, 6m	Pial arterioles	ACh↓, ADP↓	SNP↓	PKC blockade (P)
Matsunaga *et al*., 1996	dog, AL, 4w	Coronary circulation	ACh↓ adenosine↔		L-arginine (P) SOD (N)
Koltai *et al*., 1997	dog, AL, 3m	Coronary circulation	ACh↓	Sodium nitrite↔	L-arginine (N)
Angulo *et al*., 1998	rat, STZ, 8w	Hindlimb circulation	ACh↓	SNP↔	SOD (P) L-arginine (P)
Crijns *et al*., 1998	rat, STZ, 6w	Skeletal muscle arterioles	ACh↓	levchromakalim↓	aminoguanidine (N)
De Vriese *et al*., 1999	rat, STZ, 6w	Renal circulation	ACh↓	pinacidil↔ deta-NONOate↔	folate (T) COX blockade (N)

EDVD, endothelium-dependent vasodilatation; EIVD, endothelium-independent vasodilatation; AL, alloxan; STZ, streptozotocin; BB, biobred; ACh, acetylcholine; BK, bradykinin; SNP, sodium nitroprusside; GTN, glyceryltrinitrate; COX, cyclooxygenase; TP-RA, prostanoid TP receptor antagonist; TXA_2-S, thromboxane A2-synthase; PKC, protein kinase C; SOD, superoxide dismutase; ARI, aldose reductase inhibitor; DTMU, 1,3-dimethyl-2-thiourea; ↓, decreased; ↑, increased; ↔, unaltered; N, no; P, partial; T, total.

negative results were generally obtained in patients with normoalbuminuria and relatively good metabolic control (Smits *et al*., 1993; Lambert *et al*., 1996; Enderle *et al*., 1998). When microalbuminuric patients were included in the study population, impaired endothelium-dependent vasodilatation was consistently reported (Zenere *et al*., 1995; Clarkson *et al*., 1996; Lekakis *et al*., 1997; Arcaro *et al*., 1999).

Studies investigating endothelial function in animal models of type II diabetes are scarce and have yielded conflicting results. Both impaired (Sakamoto *et al*., 1998) and preserved (Bohlen & Lash, 1995) endothelium-dependent responses have been reported. Clinical studies in patients with type II diabetes are almost inevitably confounded by the high prevalence of other cardiovascular risk factors that are known to affect endothelial function. Several authors demonstrated impaired endothelium-dependent vasodilatation in patients with type II diabetes (Table 2A), but even after rigorous patient selection, mild dyslipidaemia or hypertension were often present. Although probably irrelevant for practical purposes, it thus remains unclear whether diabetes type II *per se* affects endothelial function.

Mechanisms of impaired endothelium-dependent vasodilatation in diabetes

Impaired endothelium-dependent vasodilatation may arise from several mechanisms: decreased production of one of the EDRFs, enhanced inactivation of EDRF, impaired diffusion of EDRF to the underlying smooth muscle cells, decreased responsiveness of the smooth muscle to EDRF and enhanced generation of endothelium-derived constricting factors (EDCF) (Figure 1). For each of these mechanisms, both supporting and negative evidence have been presented. Whereas differences in diabetes model and in duration or severity of diabetes undoubtedly play a role in some of the discrepancies, the type of circulation, the size of the vessel and the conditions of study may be a much more important source of disparity. This has been typically illustrated by the presence of impaired endothelium-dependent vasodilatation *in vivo* in the mesenteric circulation or in the isolated perfused mesentery of diabetic rats, and by its absence in the isolated aorta of the same animals (Fortes *et al*., 1983; Taylor *et al*., 1994b). Similarly, bradykinin-mediated vasodilatation was depressed in the hindquarters vasculature, but was normal in the kidney and mesenterium of the same diabetic rats (Kiff *et al*., 1991).

Endothelial cells from different vascular beds exhibit metabolic and structural differences and may be affected differentially by hyperglycaemia (Sobrevia & Mann, 1997). Furthermore, the mechanisms of endothelium-dependent vasodilatation may be distinct, depending on the vascular preparation of study. Although NO has been generally considered as the principal mediator of endothelium-dependent relaxations, it has become clear that EDHF may also be an important regulator of vascular tone and reactivity, especially in small resistance vessels (Félétou & Vanhoutte, 1999). Several studies demonstrated a gradient in the release of EDRFs, with a progressively increasing contribution of EDHF in the more distal vessels. The identity of EDHF has been the subject of persistent controversy. It is likely that more than one 'EDHF' exists, with substantial species and regional heterogeneity (Mombouli & Vanhoutte, 1997). Therefore, the relative contribution and the nature of the NO-independent vasodilator mechanisms may engender some of the observed discrepancies between the studies.

Most of the earlier research has concentrated on the study of isolated large conduit arteries. More recent research has shifted somewhat towards the study of isolated resistance vessels, which are of more direct relevance to the control of local blood flow. Studies of isolated perfused organs, although scarce, yield additional information, since vascular resistance and reactivity is determined by the whole circulation, including the smallest arterioles. Finally, although *in vivo* studies have limitations regarding the toxicity of certain pharmacological interventions, they allow for the study of endothelial function

Table 2. Clinical studies on endothelium-dependent vasodilatation in diabetes.

A: Observation studies

Reference	*Subjects*	*HgA1c %*	*AER*	*Ret Nr*	*AHT*	*Lipids*	*Clin Compl*	*Vascular bed*	*EDVD*	*EIVD*
Jorgensen *et al.*, 1988	38 type I 21 control	8.7–14.2	N	–	?	?	–	Forearm	FMD↓	
McVeigh *et al.*, 1992	29 type II 21 control	9.7	?	?	–	–	–	Forearm	FMD↔, ACh↓	GTN↓
Calver *et al.*, 1992	10 type I 10 control	6.7	?	?	–	chol↑	–	Forearm	ACh↔	SNP↓ verapamil↔
Smits *et al.*, 1993	11 type I 11 control	9.2	N	–	–	–	–	Forearm	FMD↔, MCh↔	SNP↔
Johnstone *et al.*, 1993	15 type I 16 control	11.9	?	?	–	–	–	Forearm	FMD↔, MCh↓	SNP↔ verapamil↔
Nitenberg *et al.*, 1993	6 type I 5 type II 7 control	7.8	?	?	controlled	HDL↓	Abn. stress test	Coronary circulation	ACh↓	ISDN↔
MacAllister *et al.*, 1995	7 type I 7 control	7.4	?	?	–	?	?	Forearm	ACh↔	
Zenere *et al.*, 1995	18 type I 16 control	7.2–7.7	N (10) Mi (8)	?	–	–	–	Femoral artery	FMD↓ (>Mi)	GTN↓ (>Mi)
Lambert *et al.*, 1996	52 type I 52 control	7.9	N	10	–	–	–	Brachial artery	FMD↔	GTN↔
Khan *et al.*, 1996	16 type I 20 control	9.6	?	–	–	HDL↓	–	Forearm	FMD↓, MCh↔	SNP↓
								Skin	FMD↔, MCh↔	SNP↔
Ting *et al.*, 1996	10 type II 10 control	7.9	?	?	–	TG↑	–	Forearm	MCh↓	
Williams *et al.*, 1996	21 type II 23 control	11	?	?	–	TG↑	–	Forearm	FMD↔, MCh↓	SNP↓ verapamil↔
Clarkson *et al.*, 1996	80 type I 80 control	9.5	Mi (5)	10	mild	chol↑ TG↑	–	Brachial artery	FMD↓	GTN↓
Lekakis *et al.*, 1997	31 type I 26 control	6.5-7.1	N (26) Mi (5)	?	–	–	–	Brachial artery	FMD↓	IDN↔N IDN↓Mi
Enderle *et al.*, 1998	17 type I 17 control	8	N	–	–	–	–	Brachial artery	FMD↔	GTN↔
Enderle *et al.*, 1998	25 type II 25 control	9.1	N (9) Mi (16)	9	–	–	7*	Brachial artery	FMD↓	GTN↔
Gazis *et al.*, 1999	48 type II 21 control	6.9	N	–	mild	–	–	Forearm	ACh↓, BK↔	SNP↔
Arcaro *et al.*, 1999	9 type I 17 control	7.9	Mi	3	–	–	–	Femoral artery	FMD↓	GTN↔

B. Intervention studies

Reference	*Patients*	*Trial design*	*Intervention*	*Vascular bed*	*EDVD*	*EIVD*
McVeigh *et al.*, 1993	23 type II	Placebo, randomized, double-blind, cross-over	Fish oil p.o. 6w	Forearm	ACh↑	GTN↔
Bijlstra *et al.*, 1995	10 type II	No placebo	Perindopril 4-8mg p.o. 6m	Forearm	FMD↑ MCh↔	SNP↔
Ting *et al.*, 1996	10 type II	No placebo	Vitamin C i.v.	Forearm	MCh↑	SNP↔ verapamil↔
O'Driscoll *et al.*, 1997	9 type I	No placebo	Enalaprilat i.v. Enalapril 20mg p.o. 4w	Forearm	ACh↑	SNP↔
Mullen *et al.*, 1998	91 type I	Placebo, randomized, double-blind, parallel	Enalapril 20mg p.o. 24w	Brachial artery	FMD↑	GTN↔
McFarlane *et al.*, 1999	20 type I	Placebo, randomized, double-blind, cross-over	Perindopril 4mg p.o. 12w	Brachial artery	FMD↔	
Gazis *et al.*, 1999	48 type II	Placebo, randomized, double-blind, parallel	Tocopherol p.o. 8w	Forearm	ACh↔ BK↔	SNP↔
Arcaro *et al.*, 1999	9 type I	Placebo, randomized, double-blind, cross-over	Captopril 75mg/ Enalapril 10mg p.o. 1w	Femoral artery	FMD↑	GTN↔
O'Driscoll *et al.*, 1999	10 type II	Placebo, randomized, double-blind, cross-over	Enalapril 20mg p.o. 4w	Forearm	ACh↑	SNP↔

EDVD, endothelium-dependent vasodilatation; EIVD, endothelium-independent vasodilatation; AER, albumin excretion rate; N, normoalbuminuria; Mi, microalbuminuria; Ret, retinopathy; AHT, arterial hypertension; FMD, flow mediated dilatation; MCh, metacholine; ACh, acetylcholine; BK, bradykinin; SNP, sodium nitroprusside; GTN, glyceryltrinitrate; ISDN, isosorbide dinitrate; ↓, decreased; ↑, increased; ↔, unaltered; *five peripheral neuropathy, two coronary artery disease of which one also peripheral vascular disease; p.o., oral; i.v., intravenous.

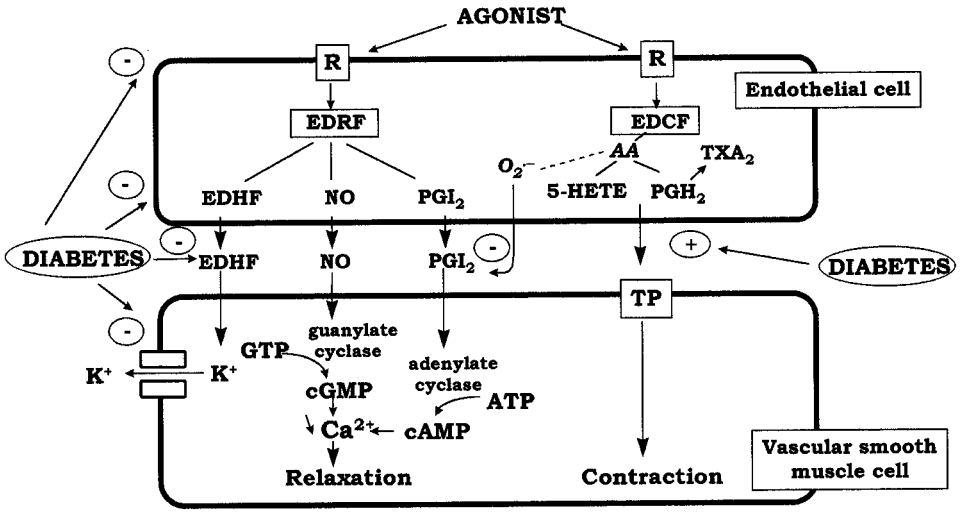

Figure 1 Mechanisms of endothelial dysfunction in diabetes. R, receptor; EDRF, endothelium-derived relaxing factor; EDHF, endothelium-derived hyperpolarizing factor; PGI_2, prostacyclin; EDCF, endothelium-derived constricting factors; TXA_2, thromboxane A_2; PGH_2, prostaglandin H_2; 5-HETE, 5-hydroxyeicosatetraenoic acid; TP, prostanoid TP receptor; $O_2^{\cdot -}$, superoxide anion.

under physiologic flow conditions and in the presence of the diabetic extracellular fluid composition. It is therefore imperative to consider evidence from all types of experimental conditions before solid conclusions can be drawn.

Signal transduction pathway Reduced expression and structural modifications of G-proteins, with reversal upon insulin treatment, have been described in diabetic rat retina (Sobrevia & Mann, 1997). Impaired ACh-induced relaxation with normal responses to bradykinin has been reported in isolated resistance vessels from patients with type I diabetes (McNally *et al*., 1994), in the forearm circulation of type II diabetes patients (Gazis *et al*., 1999) and in mesenteric and hindlimb arteries of streptozotocin (STZ)-rats (Lash & Bohlen, 1991; Taylor *et al*., 1995), suggesting an abnormality at the level of the G-proteins. However, several other studies found equally suppressed responses to different endothelium-dependent agonists (Heygate *et al*., 1995; Fulton *et al*., 1996; Costa e Forti & Fonteles, 1998; Mayhan & Patel, 1995; 1998; Mayhan, 1997) or impaired relaxation to the calcium-ionophore A23187 (Oyama *et al*., 1986; Durante *et al*., 1988; Cameron & Cotter, 1992; Fukao *et al*., 1997), making a disturbance of receptors or receptor-coupled mechanisms unlikely as a common mechanism of endothelial dysfunction.

Substrate availability Although the supply of L-arginine is not a rate-limiting factor for NO synthesis in normal circumstances, reduced availability or impaired transport or metabolism of L-arginine could be a mechanism of endothelial dysfunction in diabetic vessels. Markedly reduced serum arginine levels have been observed in diabetic rats (Pieper & Peltier, 1995; Rösen *et al*., 1996; Angulo *et al*., 1998) and have been attributed to enhanced consumption of L-arginine due to an increased NO synthesis. In accordance, an increased NO synthase activity, measured by conversion of ^{3}H-L-arginine to ^{3}H-L-citrulline, was demonstrated in diabetic rat heart endothelium (Rösen *et al*., 1996). Exogenous L-arginine (partially) restored endothelium-dependent vasodilatation in certain (Pieper & Peltier, 1995; Fulton *et al*., 1996; Matsunaga *et al*., 1996; Angulo *et al*., 1998), but not all studies (Heygate *et al*., 1995; Koltai *et al*., 1997; Mayhan *et al*., 1997). These variable results may relate to an aspecific effect of L-arginine: this amino acid is known to release insulin, which by itself may stimulate endothelium-dependent vasodilatation (MacAllister *et al*., 1995).

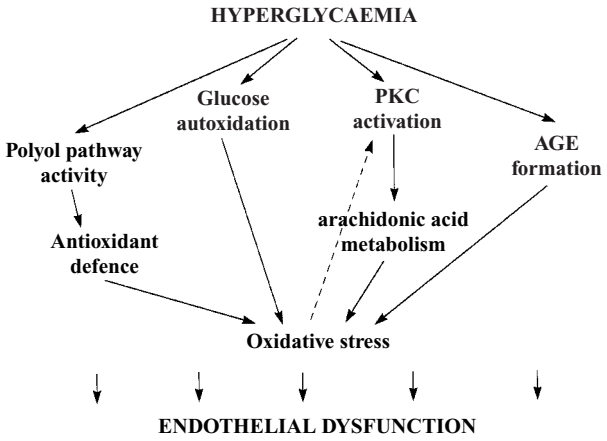

Figure 2 Outline and interactions of hyperglycaemia-induced metabolic pathways potentially involved in the pathophysiology of endothelial dysfunction.

Increased destruction of EDRF Much of the attention has focused on the extent of the endothelium-dependent vasodilatation. However, a more transient relaxation has also been reported in the aorta of diabetic rats, even though the degree of relaxation was normal (Hattori *et al*., 1991). Superoxide dismutase (SOD) restored the duration of the aortic relaxation, suggesting inactivation of EDRF by oxygen-derived free radicals. In a EDRF bioassay experiment, the perfusion of diabetic aorta produced less relaxation of a bioassay ring, as compared to control aorta. Infusion of SOD at a site proximal to the donor segment normalized the relaxations, suggesting that similar levels of EDRF are released by diabetic aorta, but that their action is attenuated by reactive oxygen species (Pieper *et al*., 1992). The role of free radicals will be more extensively discussed under the subheading 'Oxidative stress'.

EDCF Several observations implicate an overproduction of endothelium-derived vasoconstrictors, most likely prostanoids,

in the pathophysiology of endothelial dysfunction, e.g. in pial arterioles of diabetic rats *in vivo* (Mayhan *et al*., 1991) and in diabetic isolated aorta (Shimizu *et al*., 1993; Tesfamariam *et al*., 1989). Similar mechanisms may play a role after *in vitro* exposure of rabbit aorta to high glucose concentrations (Tesfamariam *et al*., 1990). These EDCFs are thought to be released together with the EDRFs and oppose their effects on the smooth muscle cells. The impaired relaxations are restored by non-specific cyclo-oxygenase blockade and prostanoid TP receptor antagonists, but not by thromboxane A_2 synthase blockers, suggesting that the culprit is a prostaglandin endoperoxide (Tesfamariam *et al*., 1989; 1990; Mayhan *et al*., 1991; Shimizu *et al*., 1993). On the other hand, cyclo-oxygenase inhibition did not restore impaired endothelium-dependent relaxations in isolated mesenteric arteries (Taylor *et al*., 1992; Diederich *et al*., 1994; Fukao *et al*., 1997), in the isolated perfused heart and kidney (Quilley *et al*., 1996; Fulton *et al*., 1996) and in the renal microcirculation *in vivo* (De Vriese *et al*., 1999), indicating that vasoactive prostanoids do not play an important contributory role to the endothelial dysfunction in these vascular beds.

EDHF Few studies have focused on the contribution of EDHF to endothelial dysfunction in diabetes. In the absence of specific inhibitors of EDHF, most of the current evidence is inevitably indirect. Decreased ACh-induced hyperpolarization and NO synthase- and cyclo-oxygenase-resistant relaxation were observed in isolated mesenteric arteries (Fukao *et al*., 1997). In the Langendorff perfused heart (Quilley *et al*., 1996), in the isolated perfused kidney (Fulton *et al*., 1996) as well as in the renal microcirculation *in vivo* (De Vriese *et al*., 1999), the NO synthase- and cyclo-oxygenase-resistant vasodilatation to bradykinin or ACh was profoundly impaired. Other authors observed a more pronounced deficit of the ACh-induced relaxations in the presence of NO synthase- and cyclo-oxygenase-blockade in mesenteric arteries (Taylor *et al*., 1992) or a decreased NO synthase-resistant ACh-induced relaxation in isolated renal arteries of diabetic rats (Dai *et al*., 1993), but they did not link their findings to an impaired EDHF-mediated influence. In the isolated rat aorta, no evidence was found for a decreased contribution of EDHF to the endothelium-dependent relaxations (Endo *et al*., 1995). Since the contribution of EDHF is most pronounced in smaller vessels, it is not surprising that evidence for a role for EDHF in diabetic endothelial dysfunction is restricted to resistance artery and whole organ studies.

Decreased responsiveness of the vascular smooth muscle to EDRF The large majority of the studies demonstrate an impaired vasodilatation to endothelium-dependent agonists in the presence of preserved responses to endothelium-independent vasodilatators. This suggests that the diabetic state does not cause a generalized reduction in the sensitivity of the smooth muscle to EDRF – at least not initially. In one of the few experimental studies where a decreased response to nitrovasodilators was observed, it was preceded by a disturbed response to ACh (Dai *et al*., 1993). An impaired response to nitrovasodilators was more frequently found in humans (McVeigh *et al*., 1992; Calver *et al*., 1992; Zenere *et al*., 1995; Williams *et al*., 1996; Clarkson *et al*., 1996; Lekakis *et al*., 1997), perhaps due to the more advanced state of the diabetes. In support of this contention, the dilatation to isosorbide dinitrate was decreased in microalbuminuric, but not in normoalbuminuric patients (Lekakis *et al*., 1997). These findings suggest that distinct mechanisms may mediate the impaired response to endothelium-independent agonists.

Interestingly, a selectively decreased responsiveness to ATP-operated potassium channel openers has been reported by several authors (Cameron & Cotter, 1992; Mayhan & Faraci, 1993; Crijns *et al*., 1998), although a preserved response was noted in other studies (Fukao *et al*., 1997; De Vriese *et al*., 1999).

Conclusion As demonstrated in this section, a broad spectrum of altered properties is potentially responsible for disordered endothelium-dependent vasodilatation in diabetes. In the aorta, impaired endothelium-dependent vasodilatation can largely be attributed to production of vasoconstrictor prostanoids and/or oxygen-derived free radicals. An important action of the latter may be the rapid destruction of NO, initially leading to an increased NO synthase activity. NO production may ultimately become compromised, perhaps by limited availability of the substrate L-arginine. In the smaller vessels, the situation is less clear-cut, and several types of interventions have failed to restore the defect. One reason may be that most of the research has focused on impaired NO-mediated vasodilatation with less concern for NO-independent vasodilatory mechanisms. Often, decreased endothelium-dependent vasodilatation was automatically reported as decreased NO-mediated vasodilatation, without knowledge of the relative contribution of the different endothelium-derived vasodilator mechanisms. As outlined above, EDHF is gaining importance as an alternative regulator of vascular tone and reactivity. This new level of understanding will hopefully give impetus to more research into NO-independent mechanisms of endothelial dysfunction, which may be especially important in small arteries.

Aetiology of endothelial dysfunction in diabetes

Although the nature of the pathogenic link between high ambient glucose concentrations and diabetic complications remains a matter of debate, hyperglycaemia is clearly recognized as the primary culprit in the pathogenesis of diabetic complications. Hyperglycaemia induces repeated acute changes in intracellular metabolism (activation of polyol pathway, activation of diacylglycerol-protein kinase C, increased oxidative stress), as well as cumulative long-term changes in the structure and function of macromolecules through formation of advanced glycation end products (AGEs). The present part of the review examines the evidence for the involvement of these pathways in the pathogenesis of endothelial dysfunction (Figure 2). The different pathways intersect at several points and potential interactions will be discussed when relevant.

Hyperglycaemia Impaired ACh-induced relaxation was reversed by chronic insulin treatment (Wang *et al*., 1993; Taylor *et al*., 1994a), but not by acute insulin administration, even though glycaemia was normalized (Wang *et al*., 1993; Bucala *et al*., 1991). Defective endothelium-dependent relaxation was restored 4 weeks after pancreatic transplantation, performed in rats after 12 weeks of diabetes (Pieper *et al*., 1998a). A close relationship between endothelial dysfunction and metabolic control was found in streptozotocin-diabetic rats in which the degree of hyperglycaemia was manipulated with subcutaneous insulin implants (Angulo *et al*., 1998). Conversely, acute exposure to high glucose concentrations induces endothelial dysfunction similar to that in diabetic animals (Tesfamariam *et al*., 1990; 1991; Tesfamariam & Cohen, 1992; Bohlen & Lash, 1993; Mayhan & Patel, 1995). Since little research on

endothelial dysfunction has been conducted in animal models of type II diabetes, it is unknown whether hyperglycaemia, in the presence of hyperinsulinaemia and insulin resistance, has the same deleterious effects on endothelial cell metabolism as in type I diabetes. In Otsuka Long-Evans Tokushima Fatty rats, endothelial dysfunction was improved by exercise training but not by food restriction, although both measures similarly improved hyperglycaemia and serum lipid levels, lessened abdominal fat content, and increased sensitivity to insulin, suggesting that the beneficial effect of exercise was unrelated to these factors (Sakamoto *et al*., 1998).

In human diabetes, the evidence is less straightforward. Glycaemic control is a predictor of micro- and macrovascular complications, although the relationship is relatively weak, especially in type II diabetes. More pronounced endothelial dysfunction was reported in type I patients with poor glycaemic control as compared with patients with better haemoglobin A_{1c} (Jorgensen *et al*., 1988). However, other studies found no correlation between haemoglobin A_{1c} values and degree of endothelium-dependent vasodilatation (Johnstone *et al*., 1993; Clarkson *et al*., 1996; Lambert *et al*., 1996; Mullen *et al*., 1998). These observations may indicate that, in human diabetes, hyperglycaemia-induced cellular alterations are substantially modulated downstream. Alternatively, the coexistence of other risk factors may be required for the full expression of the damaging effects of hyperglycaemia.

Aldose reductase In tissues that do not require insulin for cellular glucose uptake, such as the kidney, retina, nerves and blood vessels, hyperglycaemia activates the polyol pathway, resulting in the formation of sorbitol (Gabbay, 1973). Aldose reductase is the first and rate-limiting enzyme in the polyol pathway and reduces the aldehyde form of glucose to sorbitol. Several experimental and clinical studies have evidenced a link between the increased polyol pathway activity and the occurrence of chronic diabetic complications. Interestingly, only the classic target organs of diabetic complications were found to be sensitive to damage associated with elevated levels of human aldose reductase gene expression in transgenic mice carrying human aldose reductase cDNA (Giugliano *et al*., 1996). Aldose reductase inhibitors were effective in the prevention of experimental diabetic neuropathy, albuminuria and cataracts (Zenon *et al*., 1990). Consequently, aldose reductase inhibitors were tested for their ability to improve endothelial dysfunction in experimental models of diabetes. Chronic oral treatment with structurally dissimilar aldose reductase inhibitors restored abnormal endothelium-dependent vasodilatation in all (Cameron & Cotter, 1992; Tesfamariam *et al*., 1993; Otter & Chess-Williams, 1994; Quilley *et al*., 1996) but one (Taylor *et al*., 1994b) study. The mechanisms responsible for the beneficial effects of aldose reductase inhibitors have not been elucidated, but several hypotheses have been formulated. Aldose reductase utilizes NADPH for the conversion of glucose to sorbitol and may thus deplete the cellular stores of NADPH (Gabbay, 1973). Reduced NADPH is required for the functioning of many endothelial enzymes, including NO synthase and cytochrome P450, as well as for the antioxidant activity of glutathione reductase. Alternatively, a high polyol pathway flux consumes large quantities of ATP and may thus compromise the energy supply required for EDRF production (Cameron & Cotter, 1992). Aldose reductase inhibitors prevent the consumption of NADPH and energy in the polyol pathway and by virtue of this, may restore impaired EDRF production and endogenous antioxidant protection. So far, no studies have evaluated the potential beneficial effect of aldose reductase inhibitors on endothelial function in human diabetes. Although initial studies with aldose reductase inhibitors in experimental diabetic neuropathy were promising, it should be noted that these drugs have consistently failed to demonstrate a clinically meaningful improvement of diabetic neuropathy in humans (Pfeifer *et al*., 1997).

Protein kinase C Another glucose-induced alteration in cellular metabolism that may account for endothelial dysfunction is activation of protein kinase C. Hyperglycaemia causes *de novo* synthesis of diacylglycerol, leading to activation of protein kinase C -preferentially the β-isoform- , a pathway now demonstrated in all vascular tissues involved in diabetic complications (Craven *et al*., 1995; Koya & King, 1998). The consequences of protein kinase C activation are multiple, since it is involved in a variety of cellular functions (Koya & King, 1998). Of relevance to impaired responses to endothelium-dependent agonists are the activation of phospholipase A_2 with increased production of arachidonic acid metabolites, and the inhibition of Na^+-K^+-ATPase. The adverse effects of elevated glucose levels on ACh-induced relaxation of rabbit aorta and rat pial arterioles were restored by the addition of protein kinase C-inhibitors (Tesfamariam *et al*., 1991; Mayhan & Patel, 1995). In addition, the glucose-induced release of vasoconstrictor prostanoids was prevented by protein kinase C-inhibition (Tesfamariam *et al*., 1991). In experimental diabetes, protein kinase C-inhibitors improved endothelial dysfunction in pial arterioles *in vivo* (Pelligrino *et al*., 1994), but not in isolated mesenteric arteries (Diederich *et al*., 1994).

Vitamin E was reported to prevent diacylglycerol-protein kinase C-mediated vascular dysfunction in diabetes (Kunisaki *et al*., 1995), indicating a link between oxidative stress and the protein kinase C pathway.

AGEs Glucose is known to bind non-enzymatically to free amino groups on proteins or to lipids. Through a series of oxidative and non-oxidative reactions, AGEs are formed irreversibly and accumulate in tissues over time. Recently, the concept of non-enzymatic protein modification by glucose has been broadened to include a variety of reactive carbonyl compounds that are capable of AGE formation, and the term 'carbonyl stress' has been put forward (Miyata *et al*., 1999). Although AGE formation occurs during the normal ageing process, it is markedly accelerated during diabetes, as a consequence of an increase in substrate, e.g. glucose, and in the prevailing oxidant stress in this disease (Baynes & Thorpe, 1999). The pathogenicity of AGEs is related to their ability to accumulate in tissues with the formation of cross-links, and to generate oxygen-derived free radicals. In addition, the interaction of AGEs with their cellular receptors (RAGEs) may trigger sustained cellular activation and a further increase of the oxidative stress (Schmidt *et al*., 1999). Treatment with aminoguanidine, an inhibitor of AGE formation, has proven beneficial on the progression of a broad range of diabetic complications in animal models and is currently under study in human diabetes (Friedman, 1999). Interpretation of the effects of aminoguanidine may be complicated by other actions of the component, including inhibition of NO synthase (Tilton *et al*., 1993).

AGEs are known to quench NO (Bucala *et al*., 1991), but the relevance of this *in vitro* phenomenon to the *in vivo* situation has not been demonstrated. Aminoguanidine partially prevented the time-dependent progression of impaired vasodilatation to acetylcholine and nitroglycerin in

STZ-diabetes (Bucala *et al*., 1991). Since no other vasodilator responses were tested, it is unclear whether this protective effect was related to decreased NO quenching or to an aspecific improvement of vascular distensibility. Several authors found no beneficial effect of aminoguanidine on disordered endothelium-dependent vasodilatation in experimental diabetes (Hill & Ege, 1994; Pieper *et al*., 1996; Crijns *et al*., 1998). In contrast, aminoguanidine prevented diabetes-induced changes in arteriolar mechanical behaviour, as defined by decreased passive compliance and impaired myogenic reactivity of the arteriolar wall (Huijberts, *et al*., 1993; Hill & Ege, 1994). Taken together, the deleterious effects of AGE accumulation in vascular tissues are more likely related to alterations in the connective tissue composition of the microvascular wall resulting in increased tissue rigidity, rather than to functional interference with vascular smooth muscle reactivity.

Oxidative stress A considerable body of evidence implicates oxidative stress as an important pathogenic element in diabetic endothelial dysfunction. Oxidative stress is defined as an increase in the steady-state levels of reactive oxygen species and may occur as a result of increased free radical generation and/or decreased anti-oxidant defence mechanisms. Although there is controversy about the antioxidant status in diabetes, several studies have reported decreased plasma or tissue concentrations of superoxide dismutase, catalase, glutathione and ascorbic acid in both clinical and experimental diabetes (Giugliano *et al*., 1996). Diabetic aorta was found to be more sensitive to free radical exposure than normal aorta (Pieper & Gross, 1988). In addition, diabetes has been associated with an increased generation of oxygen-derived free radicals (Giugliano *et al*., 1996). Sources of reactive oxygen species in diabetes may include autoxidation of glucose (Wolff & Dean, 1987), AGE-formation and the binding of AGEs to their receptors (Yan, *et al*., 1994; Ceriello, 1999), increased substrate flux through the polyol pathway (Giugliano *et al*., 1996) and stimulation of eicosanoid metabolism (Kontos, 1987; Tesfamariam & Cohen, 1992). Oxygen-derived free radicals may impair endothelium-dependent vasodilatation through inactivation of NO or by serving as an EDCF (Rubanyi & Vanhoutte, 1986; Katusic & Vanhoutte, 1989). Acute administration of scavengers of superoxide anion, including superoxide dismutase (Hattori *et al*., 1991; Tesfamariam & Cohen, 1992; Pieper *et al*., 1996; Bohlen & Lash, 1993) and the combination of superoxide dismutase with catalase (Pieper *et al*., 1997) improved or normalized the abnormal endothelium-dependent responses in different models of diabetes and during high glucose exposure. Similarly, chronic treatment with probucol (Tesfamariam & Cohen, 1992), N-acetylcysteine (Pieper & Siebeneich, 1998b), vitamin E (Keegan *et al*., 1995; Rösen *et al*., 1996) and vitamin C (Ting *et al*., 1996) prevented the development of endothelial dysfunction in clinical and experimental diabetes. In an *in vivo* study of high glucose exposure of the mesenteric circulation, superoxide dismutase and catalase were equally or more effective than cyclo-oxygenase inhibition in restoring the impaired ACh-induced vasodilatation, suggesting that the oxygen-derived radicals produced during prostanoid synthesis, rather than the prostanoids themselves, were responsible for the endothelial dysfunction (Bohlen & Lash, 1993). In some studies, hydroxyl radical scavengers restored endothelium-dependent vasodilatation, while superoxide dismutase had less or no effect (Dai *et al*., 1993; Diederich *et al*., 1994; Pieper *et al*., 1997; Mayhan & Patel, 1998), suggesting a more important role of the hydroxyl radical in eliciting endothelial dysfunction.

In contrast, several studies failed to demonstrate a beneficial effect of antioxidant administration in resistance arteries (Heygate *et al*., 1995; Matsunaga *et al*., 1996; Palmer *et al*., 1998a,b). This may be related to the more limited contribution of NO to endothelium-dependent vasodilatation in these vessels. In the forearm circulation of patients with type II diabetes, vitamin E supplementation during 8 weeks did not improve endothelium-dependent vasodilatation (Gazis *et al*., 1999). Surprisingly, high dose vitamin E supplementation caused a further attenuation of endothelium-dependent vasodilatation in mesenteric arteries of the rat, despite a decrease of the 8-epi-prostaglandin $F_{2\alpha}$ level, an indicator of oxidative stress (Palmer *et al*., 1998a), suggesting that exaggerated antioxidant supplementation may even be deleterious.

Further studies examining the long-term effects of antioxidant supplementation will be required, before antioxidant vitamins can be recommended for the prevention of vascular complications in diabetes.

Conclusion There exist several intersections and areas of overlap between the principal mediators of glucose-induced damage to the vascular endothelium. It is therefore not surprising that correction of any of them may result in amelioration of endothelial dysfunction and the efficiency of one approach does not necessarily preclude that other mechanisms are at play as well.

Endothelial dysfunction as a therapeutic target

Recent interest has focused on strategies to reverse or retard endothelial dysfunction in order to modify the natural history of diabetic vascular disease. As outlined above, various interventions have proven effective in restoring impaired endothelium-dependent vasodilatation in certain vascular beds in animal or human diabetes. Most benefit may, however, be derived from those therapies that have an aspecific or broad beneficial action on endothelial cell metabolism.

ACE inhibitors were shown to ameliorate endothelial dysfunction in patients with diverse cardiovascular risk factors (Anderson, 1999). Similarly, ACE inhibition improved endothelium-dependent vasodilatation in type I and type II diabetes patients without affecting the response to nitrovasodilators (Bijlstra *et al*., 1995; O'Driscoll *et al*., 1997; 1999; Arcaro *et al*., 1999), but other authors did not confirm these findings (Mullen *et al*., 1998; McFarlane *et al*., 1999) (Table 2B). Although the mechanisms responsible for the beneficial effects of ACE inhibitors have not been settled, a number of potential explanations have been put forward. ACE inhibition may decrease angiotensin II-induced NADH oxidase activity and by virtue of this, decrease vascular production of superoxide anions. In addition, ACE inhibitors may stimulate basal NO production by suppression of bradykinin breakdown or perhaps by potentiation of the vascular effects of insulin (Vanhoutte *et al*., 1995).

Treatment with folate normalizes endothelial function in patients with familial hypercholesterolaemia (Verhaar *et al*., 1999). Furthermore, folate restored endothelial dysfunction during a methionine load test in healthy volunteers without affecting the rise in plasma homocysteine levels (Usui *et al*., 1999). In STZ-diabetes in the rat, folate acutely improved endothelial dysfunction in the renal microcirculation (De Vriese *et al*., 1999). If these initial observations could be confirmed in humans, folate may act as a universal tool for the prevention of vascular complications associated with different

cardiovascular risk factors. The mechanism of action of folate is at present unknown.

In addition to the development of therapies that may restore the function of the endothelium, we may have to alter our thinking on the use of treatment modalities that have the potential to destroy the endothelium, such as the commonly used balloon dilatation for vascular stenoses. Since regenerating endothelium is known to be dysfunctional (Shibano & Vanhoutte, 1994), the ultimate benefit of these therapies may be questionable.

Conclusions and future perspectives

The present communication reviews the reported studies on the pathophysiology of endothelium-dependent vasodilatation in experimental and clinical diabetes. The high number of available studies and the disparity of the findings highlight the complex pathophysiology of disordered endothelium-dependent vasodilatation in diabetes. Several metabolic pathways overlap and intersect in their adverse effects on endothelial cell homeostasis. In addition, the susceptibility of tissues to the damaging effects of hyperglycaemia may vary. Finally, the mechanisms of endothelium-dependent vasodilatation may be quite different according to the size of the vessel and its anatomical location. In the light of these considerations, it may be hazardous to extrapolate conclusions drawn in one vessel type or diabetes model to another. Such observations emphasize the importance to select clinically relevant models for future studies on endothelial dysfunction.

First, the prevalence of type II diabetes has been rising dramatically over the past few decades. Currently, diabetes type II accounts for more than 90% of the diabetic population. The natural history of vascular disease in type II diabetes may differ substantially from that in type I diabetes. Experimental research should therefore shift from STZ-diabetes to animal models of type II diabetes, as are now commonly available and increasingly better characterized (Perico & Remuzzi, 1999).

Second, experimental research has mainly focused on large conduit arteries such as the aorta and resistance vessels from the mesenteric circulation, whereas clinical research was largely conducted in the forearm circulation. It may be more relevant to study endothelial dysfunction in the typical target organs responsible for the clinical complications of diabetes, such as the circulations of the kidney, heart, retina and brain.

Third, although altered vascular reactivity and compliance *per se* may influence target organ functioning, disordered endothelium-dependent vasodilatation is primarily *a marker* of endothelial dysfunction. A particular intervention that improves endothelium-dependent vasodilatation is likely to confer commensurate benefit in other aspects of endothelial function. Nevertheless, it is imperative to link the effect on endothelium-dependent vasodilatation to a therapeutic impact on long-term target organ functioning and ultimately on survival.

The progress that has been made in the understanding of the complex pathophysiology of disordered endothelium-dependent vasodilatation in diabetes has set the stage for further investigation of therapeutic interventions to restore endothelial function. Hopefully, these 'endothelial cell replacement therapies' will have the potential to improve the dismal prognosis of diabetic vascular disease.

References

ANDERSON, T.J. (1999). Assessment and treatment of endothelial dysfunction in humans. *J. Am. Coll. Cardiol.,* **34,** 631–638.

ANGULO, J., RODRIGUEZ-MANAS, L., PEIRO, C., NEIRA, M., MARIN, J. & SANCHEZ-FERRER, C.F. (1998). Impairment of nitric oxide-mediated relaxations in anaesthetized autoperfused streptozotocin-induced diabetic rats. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **358,** 529–537.

ARCARO, G., ZENERE, B.M., SAGGIANI, F., ZENTI, M.G., MONAUNI, T., LECHI, A., MUGGEO, M. & BONADONNA, R.C. (1999). ACE inhibitors improve endothelial function in type 1 diabetic patients with normal arterial pressure and microalbuminuria. *Diabetes Care,* **22,** 1536–1542.

BAYNES, J.W. & THORPE, S.R. (1999). Role of oxidative stress in diabetic complications. A new perspective on an old paradigm. *Diabetes,* **48,** 1–9.

BHARDWAJ, R. & MOORE, P.K. (1988). Increased vasodilator response to acetylcholine of renal blood vessels from diabetic rats. *J. Pharm. Pharmacol.,* **40,** 739–742.

BIJLSTRA, P.J., SMITS, P., LUTTERMAN, J.A. & THIEN, T. (1995). Effect of long-term angiotensin-converting enzyme inhibition on endothelial function in patients with the insulin-resistance syndrome. *J. Cardiovasc. Pharmacol.,* **25,** 658–664.

BOHLEN, H.G. & LASH, J.M. (1993). Topical hyperglycemia rapidly suppresses EDRF-mediated vasodilation of normal rat arterioles. *Am. J. Physiol.,* **265,** H219–H225.

BOHLEN, H.G. & LASH, J.M. (1995). Endothelium-dependent vasodilation is preserved in non-insulin-dependent Zucker fatty diabetic rats. *Am. J. Physiol.,* **268,** H2366–H2374.

BUCALA, R., TRACEY, K.J. & CERAMI, A. (1991). Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. *J. Clin. Invest.,* **87,** 432–438.

CALVER, A., COLLIER, J. & VALLANCE, P. (1992). 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.

CAMERON, N.E. & COTTER, M.A. (1992). Impaired contraction and relaxation in aorta from streptozotocin-diabetic rats: role of polyol pathway. *Diabetologia,* **35,** 1011–1019.

CERIELLO A. (1999). Hyperglycemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. *Diabetes Nutr. Metab.,* **12,** 42–46.

CLARKSON, P., CELERMAJER, D.S., DONALD, A.E., SAMPSON, M., SORENSEN, K.E., ADAMS, M., YUE, D.K., BETTERIDGE D.J. & DEANFIELD, J.E. (1996). Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. *J. Am. Coll. Cardiol.,* **28,** 573–579.

COSTA E FORTI, A. & FONTELES, M.C. (1998). Decreased endothelium dependent relaxation (nitric oxide) in diabetic kidneys. *Horm. Metab. Res.,* **30,** 55–57.

CRAVEN, P.A., STUDER, R.K., NEGRETE, H. & DERUBERTIS, F.R. (1995). Protein kinase C in diabetic nephropathy. *J. Diabetes Complic.,* **9,** 241–245.

CRIJNS, F.R., STRUIJKER BOUDIER, H.A. & WOLFFENBUTTEL, B.H. (1998). Arteriolar reactivity in conscious diabetic rats. Influence of aminoguanidine treatment. *Diabetes,* **47,** 918–923.

DAI, F., DIEDERICH, A., SKOPEC, J. & DIEDERICH, D. (1993). Diabetes-induced endothelial dysfunction in streptozotocin-treated rats: role of prostaglandin endoperoxides and free radicals. *J. Am. Soc. Nephrol.,* **4,** 1327–1336.

DE VRIESE, A., VAN DE VOORDE, J., VANHOLDER, R. & LAMEIRE, N. (1999). Impaired endothelium-derived hyperpolarizing factor-mediated renal vasodilatory response in diabetes: restoration with folate. *J. Am. Soc. Nephrol.,* **10,** 394A.

DIEDERICH, D., SKOPEC, J., DIEDERICH, A. & DAI, F. (1994). Endothelial dysfunction in mesenteric resistance arteries of diabetic rats: role of free radicals. *Am. J. Physiol.,* **266,** H1153–H1161.

DURANTE, W., SEN, A.K. & SUNAHARA, F.A. (1988). Impairment of endothelium-dependent relaxation in aortae from spontaneously diabetic rats. *Br. J. Pharmacol.,* **94,** 463–468.

ENDERLE, M.D., BENDA, N., SCHMUELLING, R.M., HAERING, H.U. & PFOHL, M. (1998). Preserved endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy subjects. *Diabetes Care,* **21,** 271–277.

ENDO, K., ABIRU, T., MACHIDA, H., KASUYA, Y. & KAMATA, K. (1995). Endothelium-derived hyperpolarizing factor does not contribute to the decrease in endothelium-dependent relaxation in the aorta of streptozotocin-induced diabetic rats. *Gen. Pharmacol.,* **26,** 149–153.

FÉLÉTOU, M. & VANHOUTTE, P.M. (1999). The alternative: EDHF. *J. Moll. Cell. Cardiol.,* **31,** 15–22.

FORTES, Z.B., LEME, J.G. & SCIVOLETTO, R. (1983). Vascular reactivity in diabetes mellitus: role of the endothelial cell. *Br. J. Pharmacol.,* **79,** 771–781.

FRIEDMAN, E.A. (1999). Advanced glycosylated end products and hyperglycemia in the pathogenesis of diabetic complications. *Diabetes Care,* **22** (Suppl 2), B65–B71.

FUKAO, M., HATTORI, Y., KANNO, M, SAKUMA, I. & KITABATAKE, A. (1997). Alterations in endothelium-dependent hyperpolarization and relaxation in mesenteric arteries from streptozotocin-induced diabetic rats. *Br. J. Pharmacol.,* **121,** 1383–1391.

FULTON, D., MCGIFF, J.C. & QUILLEY, J. (1996). Cytochrome P450 arachidonate metabolites: deficit in diabetes mellitus? *FASEB J.,* **9,** A113.

FURCHGOTT, R.F. & VANHOUTTE, P.M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB J.,* **3,** 2007–2018.

GABBAY, K.H. (1973). The sorbitol pathway and the complications of diabetes. *N. Engl. J. Med.,* **288,** 831–836.

GAZIS, A., WHITE, D.J., PAGE, S.R. & COCKCROFT, J.R. (1999). Effect of oral vitamin E (α-tocopherol) supplementation on vascular endothelial function in type 2 diabetes mellitus. *Diabet. Med.,* **16,** 304–311.

GEBREMEDHIN, D., KOLTAI, M.Z., POGATSA, G., MAGYAR, K. & HADHAZY, P. (1988). Influence of experimental diabetes on the mechanical responses of canine coronary arteries: role of endothelium. *Cardiovasc. Res.,* **22,** 537–544.

GIUGLIANO, D., CERIELLO, A. & PAOLISSO, G. (1996). Oxidative stress and diabetic vascular complications. *Diabetes Care,* **19,** 257–267.

HATTORI, Y., KAWASAKI, H., ABE, K. & KANNO, M. (1991). Superoxide dismutase recovers altered endothelium-dependent relaxation in diabetic rat aorta. *Am. J. Physiol.,* **261,** H1086–H1094.

HEAD, R.J., LONGHURST, P.A., PANEK, R.L. & STITZEL, R.E. (1987). A contrasting effect of the diabetic state upon the contractile responses of aortic preparations from the rat and rabbit. *Br. J. Pharmacol.,* **91,** 275–286.

HEYGATE, K.M., LAWRENCE, I.G., BENNETT, M.A. & THURSTON, H. (1995). Impaired endothelium-dependent relaxation in isolated resistance arteries of spontaneously diabetic rats. *Br. J. Pharmacol.,* **116,** 3251–3259.

HILL, M.A. & EGE, E.A. (1994). Active and passive mechanical properties of isolated arterioles from STZ-induced diabetic rats. Effect of aminoguanidine treatment. *Diabetes,* **43,** 1450–1456.

HUIJBERTS, M.S., WOLFFENBUTTEL, B.H., STRUIJKER BOUDIER, H.A., CRIJNS, F.R., NIEWENHUIJZEN KRUSEMAN, A.C., POITEVIN, P. & LÉVY, B.I. (1993). Aminoguanidine treatment increases elasticity and decreases fluid filtration of large arteries from diabetic rats. *J. Clin. Invest.,* **92,** 1407–1411.

JOHNSTONE, M.T., CREAGER, S.J., SCALES, K.M., CUSCO, J.A., LEE, B.K. & CREAGER, M.A. (1993). Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation,* **88,** 2510–2516.

JORGENSEN, R.G., RUSSO, L., MATTIOLI, L. & MOORE, W.V. (1988). Early detection of vascular dysfunction in type I diabetes. *Diabetes,* **37,** 292–296.

KATUSIC, Z.S. & VANHOUTTE, P.M. (1989). Superoxide anion is an endothelium-derived contracting factor. *Am. J. Physiol.,* **257,** H33–H37.

KHAN, F., COHEN, R.A., RUDERMAN, N.B., CHIPKIN, S.R. & COFFMAN, J.D. (1996). Vasodilator responses in the forearm skin of patients with insulin-dependent diabetes mellitus. *Vasc. Med.,* **1,** 187–193.

KEEGAN, A., WALBANK, H., COTTER, M.A. & CAMERON, N.E. (1995). Chronic vitamin E treatment prevents defective endothelium-dependent relaxation in diabetic rat aorta. *Diabetologia,* **38,** 1475–1478.

KIFF, R.J., GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1991). Selective impairment of hindquarters vasodilator responses to bradykinin in conscious wistar rats with streptozotocin-induced diabetes mellitus. *Br. J. Pharmacol.,* **103,** 1357–1362.

KOLTAI, M.Z., HADHAZY, P., POSA, I., KOCSIS, E., WINKLER, G., RÖSEN, P. & POGATSA, G. (1997). Characteristics of coronary endothelial dysfunction in experimental diabetes. *Cardiovasc. Res.,* **34,** 157–163.

KONTOS, H.A. (1987). Oxygen radicals from arachidonate metabolism in abnormal vascular responses. *Am. Rev. Respir. Dis.,* **136,** 474–477.

KOYA, D. & KING, G.L. (1998). Protein kinase C activation and the development of diabetic complications. *Diabetes,* **47,** 859–866.

KUNISAKI, M., BURSELL, S.E., CLERMONT, A.C., ISHII, H., BALLAS, L.M., JIROUSEK, M.R., UMEDA, F., NAWATA, H. & KING, G.L. (1995). Vitamin E prevents diabetes-induced abnormal retinal blood flow via the diacylglycerol-protein kinase C pathway. *Am. J. Physiol.,* **269,** E239–E246.

LAMBERT, J., AARSEN, M., DONKER, A.J. & STEHOUWER, C.D. (1996). Endothelium-dependent and independent vasodilation of large arteries in normoalbuminuric insulin-dependent diabetes mellitus. *Arterioscler. Thromb. Vasc. Biol.,* **16,** 705–711.

LASH, J.M. & BOHLEN, H.G. (1991). Structural and functional origins of suppressed acetylcholine vasodilation in diabetic rat arterioles. *Circ. Res.,* **69,** 1259–1268.

LEKAKIS, J., PAPAMICHAEL, C., ANASTASIOU, H., ALEVIZAKI, M., DESSES, N., SOUVATZOGLOU, A., STAMATELOPOULOS, S. & KOUTRAS, D.A. (1997). Endothelial dysfunction of conduit arteries in insulin-dependent diabetes mellitus without microalbuminuria. *Cardiovasc. Res.,* **34,** 164–168.

MACALLISTER, R.J., CALVER, A.L., COLLIER, J., EDWARDS, M.B., HERREROS, B., NUSSEY, S.S. & VALLANCE, P. (1995). Vascular and hormonal responses to arginine: provision of substrate for nitric oxide or non-specific effect. *Clin. Sci.,* **89,** 183–190.

MATSUNAGA, T., OKUMURA, K., ISHIZAKA, H., TSUNODA, R., TAYAMA, S., TABUCHI, T. & YASUE, H. (1996). Impairment of coronary blood flow regulation by endothelium-derived nitric oxide in dogs with alloxan-induced diabetes. *J. Cardiovasc. Pharmacol.,* **28,** 60–67.

MAYHAN, W.G. (1997). Superoxide dismutase partially restores impaired dilatation of the basilar artery during diabetes mellitus. *Brain. Res.,* **760,** 204–209.

MAYHAN, W.G. & FARACI, F.M. (1993). Responses of cerebral arterioles in diabetic rats to activation of ATP-sensitive potassium channels. *Am. J. Physiol.,* **265,** H152–H157.

MAYHAN, W.G. & PATEL, K.P. (1995). Acute effects of glucose on reactivity of cerebral microcirculation: role of activation of protein kinase C. *Am. J. Physiol.,* **269,** H1297–H1302.

MAYHAN, W.G. & PATEL, K.P. (1998). Treatment with dimethylthiourea prevents impaired dilation of the basilar artery during diabetes mellitus. *Am. J. Physiol.,* **274,** H1895–H1901.

MAYHAN, W.G., PATEL, K.P. & SHARPE, G.M. (1997). Effect of L-arginine on reactivity of hamster cheek pouch arterioles during diabetes mellitus. *Int. J. Microcirc.,* **17,** 107–112.

MAYHAN, W.G., SIMMONS, L.K. & SHARPE, G.M. (1991). Mechanisms of impaired responses of cerebral arterioles during diabetes mellitus. *Am. J. Physiol.,* **260,** H319–H326.

MCFARLANE, R., MCCREDIE, R.J., BONNEY, M., MOLYNEAUX, L., ZILKENS, R., CELERMAJER, D.S. & YUE, D.K. (1999). Angiotensin converting enzyme inhibition and arterial endothelial function in adults with type 1 diabetes mellitus. *Diabet. Med.,* **16,** 62–66.

MCNALLY, P.G., WATT, P.A., RIMMER, T., BURDEN, A.C., HEARNSHAW, J.R. & THURSTON, H. (1994). Impaired contraction and endothelium-dependent relaxation in isolated resistance vessels from patients with insulin-dependent diabetes mellitus. *Clin. Sci.,* **87,** 31–36.

MCVEIGH, G.E., BRENNAN, G.M., JOHNSTON, G.D., MCDERMOTT, B.J., MCGRATH, L.T., HENRY, W.R. & ANDREWS, J.W. (1992). Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia,* **35,** 771–776.

MCVEIGH, G.E., BRENNAN, G.M., JOHNSTON, G.D., MCDERMOTT, B.J., MCGRATH, L.T., HENRY, W.R., ANDREWS, J.W. & HAYES, J.R. (1993). Dietary fish oil augments nitric oxide production or release in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia,* **36,** 33–38.

MIYATA, T., VAN YPERSELE DE STRIHOU, C., KUROKAWA, K. & BAYNES, J.W. (1999). Alterations in nonenzymatic biochemistry in uremia: origin and significance of 'carbonyl stress' in long-term uremic complications. *Kidney Int.,* **55,** 389 – 399.

MOMBOULI, J.V. & VANHOUTTE, P.M. (1997). Endothelium-derived hyperpolarizing factor(s): updating the unknown. *Trends Pharmacol. Sci.,* **18,** 252 – 256.

MULHERN, M. & DOCHERTY, J.R. (1989). Effects of experimental diabetes on the responsiveness of rat aorta. *Br. J. Pharmacol.,* **97,** 1007 – 1012.

MULLEN, M.J., CLARKSON, P., DONALD, A.E., THOMSON, H., THORNE, S.A., POWE, A.J., FURUNO, T., BULL, T. & DEANFIELD, J.E. (1998). Effect of enalapril on endothelial function in young insulin-dependent diabetic patients: a randomized double-blind study. *J. Am. Coll. Cardiol.,* **31,** 1330 – 1335.

NITENBERG, A., VALENSI, P., SACHS, R., DALI, M., APTECAR, E. & ATTALI, J. (1993). Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. *Diabetes,* **42,** 1017 – 1025.

O'DRISCOLL, G., GREEN, D., MAIORANA, A., STANTON, K., COLREAVY, F. & TAYLOR, R. (1999). Improvement in endothelial function by angiotensin converting enzyme inhibition in non-insulin-dependent diabetes mellitus. *J. Am. Coll. Cardiol.,* **33,** 1506 – 1511.

O'DRISCOLL, G., GREEN, D., RANKIN, J., STANTON, K. & TAYLOR, R. (1997). Improvement in endothelial function by angiotensin converting enzyme inhibition in insulin-dependent diabetes mellitus. *J. Clin. Invest.,* **100,** 678 – 684.

OTTER, D.J. & CHESS-WILLIAMS, R. (1994). The effects of aldose reductase inhibition with ponalrestat on changes in vascular function in streptozotocin diabetic rats. *Br. J. Pharmacol.,* **113,** 576 – 580.

OYAMA, Y., KAWASAKI, H., HATTORI, Y. & KANNO, M. (1986). Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. *Eur. J. Pharmacol.,* **131,** 75 – 78.

PALMER, A.M., GOPAUL, N., DHIR, S., THOMAS, C.R., POSTON, L. & TRIBE, R.M. (1998b). Endothelial dysfunction in streptozotocin-diabetic rats is not reversed by dietary probucol or simvastatin supplementation. *Diabetologia,* **41,** 157 – 164.

PALMER, A.M., THOMAS, C.R., GOPAUL, N., DHIR, S., ÄNGGARD, E.E., POSTON, L. & TRIBE, R.M. (1998a). Dietary antioxidant supplementation reduces lipid peroxidation but impairs vascular function in small mesenteric arteries of the streptozotocin-diabetic rat. *Diabetologia,* **41,** 148 – 156.

PELLIGRINO, D.A., KOENIG, H.M., WANG, Q. & ALBRECHT, R.F. (1994). Protein kinase C suppresses receptor-mediated pial arteriolar relaxation in the diabetic rat. *NeuroReport,* **5,** 417 – 420.

PERICO, N. & REMUZZI, G. (1999). Diabetic nephropathy: animal models of human type 2 diabetes. In *Nephropathy in type 2 diabetes*. ed. Ritz, E. & Rychlík, I. pp. 47 – 57. New York: Oxford University Press.

PFEIFER, M.A., SCHUMER, M.P., GELBER, D.A. (1997). Aldose reductase inhibitors: the end of an era or the need for different trial designs? *Diabetes,* **46** (Suppl. 2), S82 – S89.

PIEPER, G.M., ADAMS, M.B. & ROZA, A.M. (1998a). Pancreatic transplantation reverses endothelial dysfunction in experimental diabetes mellitus. *Surgery,* **123,** 89 – 95.

PIEPER, G.M. & GROSS, G.J. (1988). Oxygen free radicals abolish endothelium-dependent relaxation in diabetic aorta. *Am. J. Physiol.,* **255,** H825 – H833.

PIEPER, G.M., LANGENSTROER, P. & SIEBENEICH, W. (1997). Diabetic-induced endothelial dysfunction in rat aorta: role of hydroxyl radicals. *Cardiovasc. Res.,* **34,** 145 – 156.

PIEPER, G.M., MEI, D.A., LANGENSTROER, P. & O'ROURKE, S.T. (1992). Bioassay of endothelium-derived relaxing factor in diabetic rat aorta. *Am. J. Physiol.,* **263,** H676 – H680.

PIEPER, G.M., MOORE-HILTON, G. & ROZA, A.M. (1996). Evaluation of the mechanism of endothelial dysfunction in the genetically-diabetic BB rat. *Life Sci.,* **58,** 147 – 152.

PIEPER, G.M. & PELTIER, B.A. (1995). Amelioration by L-arginine of a dysfunctional arginine/nitric oxide pathway in diabetic endothelium. *J. Cardiovasc. Pharmacol.,* **25,** 397 – 403.

PIEPER, G.M. & SIEBENEICH, W. (1998b). Oral administration of the antioxidant, N-acetylcysteine, abrogates diabetes-induced endothelial dysfunction. *J. Cardiovasc. Pharmacol.,* **32,** 101 – 105.

QUILLEY, J., MCGIFF, J.C., MIEYAL, P., RAPACON, M. & FULTON, D. (1996). NO-independent coronary vasodilation to bradykinin in diabetes. *Hypertension,* **28,** P178.

RÖSEN, P., BALLHAUSEN, T. & STOCKKLAUSER, K. (1996). Impairment of endothelium dependent relaxation in the diabetic rat heart: mechanisms and implications. *Diabetes Res. Clin. Pr.,* **31,** S143 – S155.

RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am. J. Physiol.,* **250,** H822 – H827.

SAKAMOTO, S., MINAMI, K., NIWA, Y., OHNAKA, M., NAKAYA, Y., MIZUNO, A., KUWAJIMA, M. & SHIMA, K. (1998). Effect of exercise training and food restriction on endothelium-dependent relaxation in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous NIDDM. *Diabetes,* **47,** 82 – 86.

SCHMIDT, A.M., YAN, S.D., WAUTIER, J.L. & STERN, D. (1999). Activation of receptor for advanced glycation end products. A mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ. Res.,* **84,** 489 – 497.

SHIBANO, T. & VANHOUTTE, P.M. (1994). Involvement of 5-HT2 receptors in chronic endothelial dysfunction after balloon injury of porcine coronary arteries. *Circulation,* **89,** 1776 – 1785.

SHIMIZU, K., MURAMATSU, M., KAKEGAWA, Y., ASANO, H., TOKI, Y., MIYAZAKI, Y., OKUMURA, K., HASHIMOTO, H. & ITO, T. (1993). Role of prostaglandin H_2 as an endothelial-derived contracting factor in diabetic state. *Diabetes,* **42,** 1246 – 1252.

SMITS, P., KAPMA, J., JACOBS, M., LUTTERMAN, J. & THIEN, T. (1993). Endothelium-dependent vascular relaxation in patients with type I diabetes. *Diabetes,* **42,** 148 – 153.

SOBREVIA, L. & MANN, G.E. (1997). Dysfunction of the endothelial nitric oxide signalling pathway in diabetes and hyperglycaemia. *Exp. Physiol.,* **82,** 423 – 452.

TAYLOR, P.D., GRAVES, J.E. & POSTON, L. (1995). Selective impairment of acetylcholine-mediated endothelium-dependent relaxation in isolated resistance arteries of streptozotocin-induced diabetic rat. *Clin. Sci.,* **88,** 519 – 524.

TAYLOR, P.D., MCCARTHY, A.L., THOMAS, C.R. & POSTON, L. (1992). Endothelium-dependent relaxation and noradrenaline sensitivity in mesenteric resistance arteries of streptozotocin-induced diabetic rats. *Br. J. Pharmacol.,* **107,** 393 – 399.

TAYLOR, P.D., OON, B.B., THOMAS, C.R. & POSTON, L. (1994a). Prevention by insulin treatment of endothelial dysfunction but not enhanced noradrenaline-induced contractility in mesenteric resistance arteries from streptozotocin-induced diabetic rats. *Br. J. Pharmacol.,* **111,** 35 – 41.

TAYLOR, P.D., WICKENDEN, A.D., MIRRLEES, D.J. & POSTON, L. (1994b). Endothelial function in the isolated perfused mesentery and aortae of rats with streptozotocin-induced diabetes: effect of treatment with the aldose reductase inhibitor, ponalrestat. *Br. J. Pharmacol.,* **111,** 42 – 48.

TESFAMARIAM, B., BROWN, M.L. & COHEN, R.A. (1991). Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase C. *J. Clin. Invest.,* **87,** 1643 – 1648.

TESFAMARIAM, B., BROWN, M.L., DEYKIN, D. & COHEN, R.A. (1990). Elevated glucose promotes generation of endothelium-derived vasoconstrictor prostanoids in rabbit aorta. *J. Clin. Invest.,* **85,** 929 – 932.

TESFAMARIAM, B.& COHEN, R.A. (1992). Free radicals mediate endothelial cell dysfunction caused by elevated glucose. *Am. J. Physiol.,* **263,** H321 – H326.

TESFAMARIAM, B., JAKUBOWSKI, J.A. & COHEN, R.A. (1989). Contraction of diabetic rabbit aorta caused by endothelium-derived PGH_2-TxA_2. *Am. J. Physiol.,* **257,** H1327 – H1333.

TESFAMARIAM, B., PALACINO, J.J., WEISBROD, R.M. & COHEN, R.A. (1993). Aldose reductase inhibition restores endothelial cell function in diabetic rabbit aorta. *J. Cardiovasc. Pharmacol.,* **21,** 205 – 211.

THE DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP/EPIDEMIOLOGY OF DIABETES INTERVENTIONS AND COMPLICATIONS RESEARCH GROUP (2000). Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *N. Eng. J. Med.,* **342,** 381 – 389.

TILTON, R.G., CHANG, K., HASAN, K.S., SMITH, S.R., PETRASH, J.M., MISKO, T.P., MOORE, W.M., CURRIE, M.G., CORBETT, J.A., MCDANIEL, M.L. & WILLIAMSON, J.R. (1993). Prevention of diabetic vascular dysfunction by guanidines. Inhibition of nitric oxide synthase versus advanced glycation end-product formation. *Diabetes,* **42,** 221 – 232.

TING, H.H., TIMIMI, F.K., BOLES, K.S., CREAGER, S.J., GANZ, P. & CREAGER, M.A. (1996). Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. *J. Clin. Invest.,* **97,** 22 – 28.

USUI, M., MATSUOKA, H., MIYAZAKI, H., UEDA, S., OKUDA, S. & IMAIZUMI, T. (1999). Endothelial dysfunction by acute hyperhomocyst(e)inaemia: restoration by folic acid. *Clin. Sci.,* **96,** 235–239.

VANHOUTTE, P.M., BOULANGER, C.M. & MOMBOULI, J.V. (1995). Endothelium-derived relaxing factors and converting enzyme inhibition. *Am. J. Cardiol.,* **76,** 3E–12E.

VERHAAR, M.C., WEVER, R.M., KASTELEIN, J.J., VAN LOON, D., MILSTIEN, S., KOOMANS, H.A. & RABELINK, T.J. (1999). Effects of oral folic acid supplementation on endothelial function in familial hypercholesterolemia: a randomized placebo-controlled trial. *Circulation,* **100,** 335–338.

WAKABAYASHI, I., HATAKE, K., KIMURA, N., KAKISHITA, E. & NAGAI, K. (1987). Modulation of vascular tonus by the endothelium in experimental diabetes. *Life Sci.,* **40,** 643–648.

WANG, Y., BROOKS, D.P. & EDWARDS, R.M. (1993). Attenuated glomerular cGMP production and renal vasodilation in streptozotocin-induced diabetic rats. *Am. J. Physiol.,* **264,** R952–R956.

WHITE, R.E. & CARRIER, G.O. (1986). Supersensitivity and endothelium dependency of histamine-induced relaxation in mesenteric arteries of diabetic rats. *Pharmacology,* **33,** 34–38.

WILLIAMS, S.B., CUSCO, J.A., RODDY M., JOHNSTONE, M.T., CREAGER, M.A. (1996). Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J. Am. Coll. Cardiol.,* **27,** 567–574.

WOLFF, S.P. & DEAN, R.T. (1987). Glucose autoxidation and protein modification. The potential role of oxidative glycosylation in diabetes. *Biochem. J.,* **245,** 243–250.

YAN, S.D., SCHMIDT, A.M., ANDERSON, G.M., ZHANG, J., BRETT, J., ZOU, Y.S., PINSKY, D. & STERN, D. (1994). Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J. Cell. Biol.,* **269,** 9889–9897.

ZENERE, B.M., ARCARO, G., SAGGIANI, F., ROSSI, L., MUGGEO, M. & LECHI, A. (1995). Noninvasive detection of functional alterations of the arterial wall in IDDM patients with and without microalbuminuria. *Diabetes Care,* **18,** 975–982.

ZENON, G.J., ABOBO, C.V., CARTER, B.L. & BALL, D.W. (1990). Potential use of aldose reductase inhibitors to prevent diabetic complications. *Clin. Pharm.,* **9,** 446–457.

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