Review

Endothelial dysfunction and pathogenesis of diabetic angiopathy

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Abstract

Objective and Methods: To review, from the clinical perspective, the contribution of dysfunction of the vascular endothelium to the pathogenesis of diabetic micro- and macroangiopathy. Results: Available data indicate that endothelial dysfunction in diabetes complicated by micro- or macroalbuminuria (renal microangiopathy) is generalised. The close linkage between microalbuminuria and endothelial dysfunction is an attractive explanation for the fact that microalbuminuria is a risk marker for atherosclerotic cardiovascular disease in diabetes. Endothelial dysfunction precedes the occurrence of even early diabetic microangiopathy. However, it is not clear whether endothelial dysfunction is a feature of the diabetic state per se or whether additional factors are required to induce endothelial dysfunction given the presence of diabetes. Convincing data from animal and in vitro models exist to indicate that endothelial dysfunction in diabetes may be related to hyperglycaemic pseudohypoxia, activation of protein kinase C, increased expression of transforming growth factor-β and vascular endothelial growth factor, non-enzymatic glycation, oxidative stress, activation of the coagulation cascade, increased expression of tumour necrosis factor-α, and high levels of insulin and insulin precursor molecules. However, the importance of these proposed mechanisms have not yet been extensively assessed in diabetes in man. Conclusions: Endothelial dysfunction plays a key role in the pathogenesis of diabetic angiopathy in man. The biochemical basis of endothelial dysfunction in diabetic man, however, has yet to be fully elucidated.

Keywords: Diabetes; Human; Endothelium; Diabetic angiopathy; Hyperglycemia; Insulin; Non-enzymatic glycation

1. Introduction

Morbidity and mortality in diabetes mellitus are caused mainly by its vascular complications: micro- and macroangiopathy. Diabetic retinopathy and nephropathy are the hallmarks of microangiopathy, with blindness and renal failure as their ultimate consequences. Macroangiopathy of the vasa nervorum is important in the pathogenesis of diabetic neuropathy. Macroangiopathy in diabetes consists mainly of an accelerated form of atherosclerosis. This affects all clinically important sites (i.e., the coronary, the carotid and the peripheral arteries), thus increasing the risk of myocardial infarction, stroke, intermittent claudication and ischaemic gangrene.

Both in insulin-dependent (IDDM) and in non-insulin-dependent diabetes (NIDDM), the presence of nephropathy, even in its early stages (so-called 'microalbuminuria'), identifies a group of patients at very high risk of developing severe complications (i.e., proliferative retinopathy, renal insufficiency, and cardiovascular disease) [1,2]. On the other hand, about 50% of IDDM patients never develop diabetic nephropathy—i.e., they appear 'protected' (reviewed in Refs. [3,4]). In other words, the risk of vascular disease is not distributed equally among diabetic patients; subgroups exist with a relatively normal versus a very high risk of cardiovascular disease.

The pathogenesis of the vascular complications of diabetes is controversial. On the basis of experimental data, various models have been proposed, but whether these models are applicable to (N)IDDM in humans is not clear [3].

We shall here examine three issues with regard to the contribution of dysfunction of the vascular endothelium to the pathogenesis of diabetic angiopathy. First, can endothe-
1. Why does endothelial dysfunction occur?

Endothelial dysfunction can adapt to temporal and local requirements. Examples are the release of products such as nitric oxide and t-PA after stimulation, and gene induction (e.g., of E-selectin) after exposure to inflammatory mediators. Dysfunction of the endothelium can be considered present when its properties, either in the basal state or after stimulation, have changed in a way that is inappropriate with regard to the preservation of organ function. For example, basement membrane synthesis may be altered, resulting in changes in cell–matrix interactions. Vascular permeability may increase, as may vascular tone. Finally, the endothelium may lose its antithrombotic and profibrinolytic properties and may instead acquire prothrombotic and antifibrinolytic properties. Such alterations do not necessarily occur simultaneously. Furthermore, they may differ according to the nature of the injury and may depend on the intrinsic properties of the endothelium (e.g., venous versus arterial versus microvascular endothelium). Therefore, endothelial dysfunction is not a discrete entity, nor does it signify a gold standard for its measurement exist.

2. What is endothelial dysfunction?

The endothelium is an important locus of control of vascular and renal functions. It actively regulates vascular tone and permeability, the balance between coagulation and fibrinolysis, the composition of the subendothelial matrix, the extravasation of leukocytes, and the proliferation of vascular smooth muscle and renal mesangial cells. To carry out these functions, the endothelium produces components of the extracellular matrix and a variety of regulatory mediators, such as nitric oxide, prostanoids, endothelin, angiotensin II, tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWf), adhesion molecules and cytokines.

Normally, the endothelium actively decreases vascular tone, a process in which nitric oxide plays a key role [6]. It closely regulates vascular permeability to nutrients, hormones and other macromolecules [7], and leukocytes [8]. In addition, endothelial cells normally inhibit platelet adhesion and aggregation by producing prostacyclin, nitric oxide and eicosanoides, limit activation of the coagulation cascade by the thrombomodulin–protein C and the hepatic sulphate–antithrombin III interactions, and regulate fibrinolysis by producing t-PA and its inhibitor, PAI-1 [9].

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3. Measurement of endothelial dysfunction in humans

There are no direct methods of measuring endothelial function in humans. It has been proposed that estimates of different types of endothelial dysfunction may be obtained indirectly by measuring endothelium-dependent vasodilation and plasma levels of endothelium-derived regulatory proteins [4–6,10–15]. This approach is summarised in Table 1.

On the one hand, the use of such estimates of endothelial dysfunction is supported by many studies showing high levels of endothelium-derived regulatory proteins and impaired endothelium-dependent vasodilation in patients with diseases that involve injury to the endothelium, such as atherosclerosis, pre-eclampsia and vasculitis [5,6]. Moreover, prospective studies have shown that, in various circumstances, subjects with high plasma levels of t-PA, PAI-1, vWF and endothelin have an adverse cardiovascular prognosis [10–15]. On the other hand, it must be emphasised that a number of unproven assumptions are
endothelial dysfunction (Table 1). First, altered function is not necessarily equivalent to dysfunction. Second, high plasma levels of endothelium-derived products may reflect increased synthesis, decreased clearance, or in some cases, increased extracellular matrix turnover; data on the latter two processes are scarce. In the case of vWF, comparison with levels of vWF-propeptide, which is co-secreted with vWF but cleared through a different pathway, may provide important information on the relative importance of synthesis versus clearance in determining vWF levels under various circumstances. Third, it is likely that the transcapillary escape rate of albumin is determined not only by the endothelium, but also by the biochemical and biophysical properties of the extracellular matrix and by haemodynamic forces. Fourth, other cell types may contribute to plasma levels. For example, PAI-1 can be produced not only by endothelial cells, but also by hepatocytes, adipocytes and vascular smooth muscle cells [16]. Fifth, as the surface area and the synthetic capacity of the microvascular endothelium are much larger than that of large-vessel endothelium, many of these estimates of endothelial dysfunction presumably reflect dysfunction at the level of small resistance arteries, capillaries or venules, while clinical events such as myocardial infarction are caused by thrombosis of large conduit arteries. Finally, it is unclear whether the prognostic value of endothelium-derived regulatory proteins is due to their specific functions (e.g., enhancement of platelet adhesion and factor VIII availability in the case of vWF [17]) or to the fact that elevated levels of any endothelium-derived regulatory protein parallel impairment of other endothelial functions (i.e., behave as a ‘marker’).

In conclusion, reasonable but not perfect estimates exist for assessing endothelial function in vivo in humans. The most extensive experience has been gained with two particular estimates: endothelium-dependent vasodilation and plasma von Willebrand factor level.

4. Endothelial dysfunction, microalbuminuria and cardiovascular disease in diabetes mellitus

If the endothelium is a target of the diabetic milieu, endothelial dysfunction might ensue. Endothelial dysfunction is thought to play an important role in the pathogenesis of (non-diabetic) glomerulosclerosis and atherosclerosis [18,19]. A considerable body of evidence in humans indicates that endothelial dysfunction is closely associated with the development of diabetic retinopathy, nephropathy and atherosclerosis, both in IDDM and in NIDDM [3,4]. Thus, endothelial dysfunction, as an early phenomenon, may explain the typical association between albuminuria and extrarenal complications [4].

In IDDM, microalbuminuria (early nephropathy) and macroalbuminuria (advanced nephropathy) are accompa-
with this hypothesis. First, early (uncomplicated) IDDM is accompanied by dilatation, not constriction, of small [30,31] and large [32] blood vessels, and an increase in microvascular blood flow, both in humans and in animal models [30,31]. Studies in humans that carefully stratified patients according to the presence or absence of a normal urinary albumin excretion rate concluded that, in reasonably well-controlled IDDM, endothelium-dependent and -independent vasodilation of resistance and conduit arteries was neither impaired nor enhanced [22,32–34].

Of note, the issue of whether, in IDDM, nitric oxide synthesis and/or action are increased, normal or decreased is an area where animal and in vitro models appear to differ widely from data obtained in humans [3]. Thus, in several animal models of IDDM, vasodilation was shown to be related to an increase in the synthesis and action of nitric oxide [31,35]. On the other hand, experimental diabetes or hyperglycaemia appeared, under certain circumstances, to be associated with a decrease of endothelium-dependent vasodilation [36,37]. As argued above, there is at present no clear evidence in favour of either of these hypotheses in human IDDM, and the mediators responsible for the vasodilation typical of early human IDDM remain to be identified. In this regard, an increased synthesis of vasodilator prostanoids has been proposed as a mediator of vasodilation in IDDM, but studies investigating this issue in man have produced conflicting results (reviewed in Ref. [5]).

In conclusion, the occurrence of endothelial dysfunction in IDDM signifies a high risk of developing severe micro- and macroangiopathy. The diabetic state itself predisposes to the development of, but is not sufficient to cause, endothelial dysfunction. Other factors, genetic or environmental, are likely to play a role in determining which IDDM patients go on to develop aggressive angiopathy and which do not [38,39]. We hypothesise that variability among individuals in endothelial vulnerability to injury may be one such factor.

5.2. Non-insulin-dependent diabetes mellitus

Several factors complicate an analysis of the role of endothelial dysfunction in the angiopathy of NIDDM. First, the natural history of NIDDM has been much less well described than that of IDDM, in part because the time of onset of NIDDM, which develops slowly and insidiously, is often unclear. Second, in terms of cardiovascular risk factors, NIDDM is a much more complex disease than is IDDM. IDDM in white subjects is typically a disease of acute onset, often before the age of 30. After the acute phase, it is usually characterised by hyperglycaemia and peripheral hyperinsulinaemia, but blood pressure and blood lipid levels are mostly normal. In contrast, NIDDM in white subjects is a disease of the middle-aged and the elderly, which typically occurs in the context of a cluster of cardiovascular risk factors, notably obesity, hypertension, high triglyceride levels, low high-density lipoprotein (HDL) cholesterol levels, abnormal low-density lipoprotein composition, hyperinsulinaemia and impaired insulin-stimulated glucose utilisation (i.e., insulin resistance)—the so-called ‘insulin resistance syndrome’ [40]. (We shall use ‘insulin resistance’ in a restricted sense—i.e., limited to insulin’s effects on glucose utilisation.)

Therefore, the definition of ‘early, uncomplicated NIDDM’ is problematic. Nevertheless, endothelial dysfunction can occur in patients with normal urinary albumin excretion [4,23,41]. In a 3-year prospective study, high levels of vWF in patients with normal urinary albumin excretion were associated with an increased risk of developing microalbuminuria [23]. Endothelium-dependent vasodilation has not yet been extensively studied in NIDDM and may [42,43] or may not [44,45] be specifically impaired. Plasma levels of endothelin [46] and of PAI-1 [47] may be elevated in uncomplicated NIDDM, although it is not known to what extent these peptides are endothelium-derived in diabetic patients.

Insulin resistance usually precedes the development of NIDDM [40]. Evidence is accumulating that insulin resistance and endothelial dysfunction are linked. First, the presence of the insulin resistance syndrome is associated with slightly elevated levels of vWF even in the absence of NIDDM [48]. Second, insulin-induced vasodilation, which is at least in part mediated by endothelium-derived nitric
oxide [49,50], is impaired in non-diabetic obese subjects [51], the classic model of insulin resistance. Third, obesity in men is associated with high plasma levels of endothelin, which decrease after weight loss [52].

Thus, insulin resistance is associated with endothelial dysfunction, and it is commonly thought that insulin resistance or the metabolic defects with which it is associated cause endothelial dysfunction [40]. However, the reverse hypothesis, that insulin resistance is caused by endothelial dysfunction, deserves serious consideration. Kinetic studies of insulin action indicate that, before insulin binds to its receptor on muscle cells, there is a rate-limiting step [53], which may in part reflect transendothelial insulin transport [54–57] and in part insulin-induced microvascular dilation [51], although the latter mechanism is by no means undisputed [58,59]. Nevertheless, it is interesting to note that endothelial dysfunction might contribute not only to insulin resistance, but also to the insulin resistance syndrome [60]. Specifically, loss of endothelium-dependent vasodilation and increased synthesis of vasoconstrictors might contribute to hypertension, and loss of endothelium-bound lipoprotein lipase activity to dyslipidaemia, both typical of the insulin resistance syndrome. Clearly, these ideas are not incompatible: insulin resistance may be both cause and consequence of endothelial dysfunction.

In conclusion, as in IDDM, endothelial dysfunction in NIDDM is closely linked to the occurrence of severe angiopathy. We hypothesise that it precedes and contributes to the development not only of angiopathy but also of the insulin resistance syndrome itself (including NIDDM).

6. What causes endothelial dysfunction in diabetes?

6.1. General outline

In IDDM, it is likely that endothelial dysfunction is caused by the metabolic consequences of impaired pancreatic functioning. In NIDDM this is less clear. For example, in certain forms of inherited NIDDM (e.g., those characterised by a decreased glucose transport and phosphorylation [61]) metabolic dysregulations in peripheral tissues may put an increased demand on both the pancreas and the vascular system, and endothelial dysfunction might thus develop in parallel to, rather than as a consequence of, hyperglycaemia. Notwithstanding these considerations, it is generally assumed that impairment of glucose and insulin metabolism may cause and/or aggravate endothelial dysfunction. Endothelial cells exposed to high glucose in vitro increase the production of extracellular matrix components, such as collagen and fibronectin, and of procoagulant proteins, such as tissue factor and von Willebrand factor, and show decreased proliferation, migration and fibrinolytic potential [62], and increased programmed cell death (apoptosis) [63].

Various hypotheses have been put forward to explain the adverse effects of diabetes on the vascular system via an effect of hyperglycaemia on vascular cells, in particular the endothelium. Several concentrate on a glucose-induced impairment of intracellular metabolic regulation. According to these hypotheses, possible targets of the dysregulation caused by elevated cellular glucose concentrations include: alterations in the cellular redox state by an altered NADH/NAD+ ratio; changes in the regulation of protein tyrosine kinases, the activity of which is influenced by the redox state of their sulphhydryl groups; dysregulation of protein kinase C (PKC), in particular the PKC-βII isoform; and the accumulation of sorbitol. An additional mechanism that may link hyperglycaemia to altered function of vascular cells is the formation of degenerated adducts between glucose and the basic amino acids lysine and arginine, the so-called ‘advanced glycation end-products’ or AGEs. Furthermore, high glucose is believed to influence endothelial cells indirectly by the synthesis of growth factors in adjacent cells, in particular transforming growth factor-β (TGFβ) and vascular endothelial growth factor (VEGF), and by acting on the coagulation cascade, which generates thrombin, a potent cell activator, and fibrin, which may also have stimulatory effects on endothelial function.

Poor glycaemic control increases the risk of diabetic micro- and macroangiopathy, but the relation is relatively weak, especially in NIDDM. This indicates either that hyperglycaemia-associated biochemical alterations in humans can be sufficiently modulated ‘downstream’ to account for large differences in clinical expression of angiopathy at similar levels of hyperglycaemia, or that hyperglycaemia is necessary but not sufficient to cause severe diabetic angiopathy, thus implicating non-glucose-mediated mechanisms.

Diabetes in humans is typically characterised by hyperglycaemia and by hyperinsulinaemia. In IDDM, this is caused by treatment with exogenous insulin; in untreated NIDDM, hyperinsulinaemia is caused by the pancreatic response to insulin resistance. As insulin resistance usually precedes the occurrence of NIDDM, NIDDM patients will have been exposed to long periods of hyperinsulinaemia before the onset of hyperglycaemia. Finally, NIDDM patients have high levels of molecules that arise as a byproduct of insulin synthesis and secretion, notably intact proinsulin, proinsulin split products, and C-peptide. In contrast, levels of these peptides are (abnormally) low in IDDM. Thus it is pertinent to examine to what extent levels of insulin and insulin precursors may affect vascular function.

NIDDM is often accompanied by obesity, dyslipidaemia and hypertension (as part of the insulin resistance syndrome). Recent studies indicate that cytokines, in particular tumour necrosis factor-α (TNF-α), may have a role in the pathogenesis of the insulin resistance syndrome, and we shall examine the implications of this finding for
vascular disease in relation to the functioning of the endothelium.

6.2. Hyperglycaemic pseudohypoxia and protein kinase C (PKC) activation

Hyperglycaemia increases the intracellular NADH/NAD\(^+\) ratio, leading to a cytosolic redox imbalance. This is related to an increased activity of the sorbitol pathway (glucose \(\rightarrow\) sorbitol \(\rightarrow\) fructose, catalysed by aldose reductase and sorbitol dehydrogenase, respectively), and to an increased rate of glycolysis. An increased NADH/NAD\(^+\) ratio has been shown to be present in experimental diabetes, and to mimic the effects of tissue hypoxia. This phenomenon has therefore been termed hyperglycaemic pseudohypoxia [31]. An increased NADH/NAD\(^+\) ratio alters various interrelated biochemical pathways resulting in changes in cellular function. There are increases in 1,2-diacyl-sn-glycerol [DAG], PKC activity, eicosanoids, long-chain fatty acids (as esters of coenzyme A or of carnitine), superoxide anion and nitric oxide, and a decrease in Na\(^+\)/K\(^-\)-ATPase.

A hyperactive sorbitol pathway may deplete the cellular NADPH pool, which is required for nitric oxide generation and to replenish glutathione. Hyperglycaemia also reduces the activity of the pentose phosphate pathway in endothelial cells, further impairing NADPH generation under conditions of increased demand [64]. As a consequence, oxidants may decrease the cellular glutathione level, which may in turn affect the activities of protein tyrosine kinases [65]. Activation of transcription factors by reactive oxygen species or by phosphorylation of tyrosine or serine/threonine protein residues by PKC or PKC-activated kinases plays a pivotal role in gene regulation, and may be important in modulating gene expression of vascular cells (e.g., to increase the synthesis of matrix components). As discussed below, some of these changes are accentuated by AGEs.

The specific biochemical consequences of an altered redox state may be time- and tissue-dependent. For example, prostaglandin synthesis is increased, but whether vasoconstrictor or vasodilator prostaglandins predominate appears to depend on the tissue investigated (e.g., aorta versus kidney). Nitric oxide synthesis can be enhanced or decreased, depending on the model studied [3]. In contrast, IDDM in humans appears not to be accompanied by an increased synthesis and/or action of nitric oxide (see Section 5.1). Activation of PKC also differs between tissues [31,66]; the PKC\(\beta\)-II isofrom (which in particular is present in vascular cells) appears preferentially affected [67,68].

PKC activation can potentially explain many of the vascular abnormalities observed in diabetes [66–69]. In animal and in vitro models, it can affect endothelial, vascular smooth muscle and mesangial cell functions, including the regulation of permeability, contractility, blood flow and basement membrane synthesis. PKC activation can modulate the actions of hormones, growth factors, and ion channels such as the sodium-proton antiport, a key regulator of intracellular pH, cell volume, growth, differentiation and contractility. PKC activation in diabetes is related to increases in intracellular DAG either from membrane-associated phosphatidyl inositol (4,5)-biphosphate or (perhaps mainly) from de novo synthesis through increased glycolysis. In streptozotocin-induced diabetes in the rat (a model of IDDM), an oral inhibitor of the PKC\(\beta\) isoforms ameliorated vascular dysfunction [68]. Interestingly, vitamin E can inhibit glucose-induced PKC\(\beta\)-II activation [70], suggesting a link between oxidative stress and PKC activation.

Nevertheless, the relevance of these biochemical alterations has yet to be established in humans in vivo [3,71]. The hyperglycaemic pseudohypoxia and PKC models per se also do not provide an explanation for the clinically evident differences, in the face of similar levels of hyperglycaemia, in the propensity of patients to develop diabetic angiopathy.

6.3. Transforming growth factor-\(\beta\) (TGF\(\beta\)) and vascular endothelial growth factor (VEGF)

In diabetes TGF\(\beta\), in particular the TGF\(\beta\)1 form, is overexpressed in renal tissue [72–74]. Because the TGF\(\beta\)1 gene is strongly activated by PKC [75], it is plausible that the induction of TGF\(\beta\) by hyperglycaemia is mediated by PKC activation. TGF\(\beta\) stimulates the accumulation of matrix proteins by enhancing, in mesangial cells and other types of renal cells, the synthesis of various matrix proteins, such as type I and IV collagens, fibronectin and proteoglycans [72,76–78], and by reducing their proteolysis [72,79]. At the same time, TGF\(\beta\) inhibits the proliferation of various cell types including glomerular endothelial cells [76]. This may reduce the ability of endothelial cells to regenerate after injury.

The enhanced TGF\(\beta\) expression and the simultaneously occurring increased synthesis of matrix proteins in hyperglycaemic animals were ameliorated after normalisation of the plasma glucose concentration by insulin treatment [73,74]. These observations strongly suggest an important role for TGF\(\beta\) in the matrix alterations in the kidney glomerulus, and possibly also in other microvessels. Such structural alterations may contribute to diabetic glomerulosclerosis.

Another growth factor—vascular endothelial growth factor (VEGF)—was found to be elevated in the vitreous of the eyes of patients with proliferative diabetic retinopathy, as compared to diabetic patients without active ocular neovascularisation [80–82]. Increased expression of VEGF by retinal cells was also demonstrated [83,84]. VEGF stimulates the proliferation of endothelial cells selectively, and is a potent angiogenic factor in vivo. It is now generally thought to be involved in the proliferative phase of diabetic retinopathy. This suggestion is underscored by...
the observation that hypoxic areas are widespread in proliferative retinopathy, and that hypoxia is a strong stimulus for enhanced VEGF synthesis in many cells [85,86]. A role of VEGF in ocular neovascularisation is also supported by recent studies in monkeys showing that VEGF injection in the vitreous of the eye induces vascular changes in the iris and eventually neovascularisation and neovascular glaucoma [84].

A link between elevated VEGF expression and hyperglycaemia [87] but also with hypoglycaemia [88] has been reported. However, this does not explain why VEGF is not elevated in diabetic patients without proliferative retinopathy. A more likely explanation may be that, after an initial (period of) injury, hypoxia occurs in areas of the retina, after which cells start to produce increased amounts of VEGF. It should also be noted that TGFβ [89] and insulin-like growth factor-I [90] have been reported to enhance VEGF production in fibroblastoid and epithelial cells, and in retinal cells, respectively. Hence it is possible that various stimuli may act in an additive or synergistic way to increase VEGF production. This issue needs further investigation, as does the role of VEGF in extra-ocular complications of diabetes.

6.4. Non-enzymatic glycation

High concentrations of glucose accelerate the spontaneous formation of glucose adducts to proteins and other amine-containing molecules. Such early non-enzymatic glycation products are reversible; glycohaemoglobin is the best-known example. These so-called ‘Amadori products’ become converted to irreversibly modified cross-linked condensation products of glucose and lysine or arginine residues, so-called ‘advanced glycation end-products’ (AGEs) [91,92]. Irreversible AGE formation is a slow and complex process in which glycation, glycoxidation and auto-oxidative glycosylation are thought to occur [92,93]. AGEs have been encountered in vivo in many different proteins, such as collagen, albumin and plasma lipoproteins [91,92]. They are also present on red blood cells [94]. AGEs represent a mixture of different adducts, the precise structure of only a few of which has been elucidated. AGE formation normally occurs at a low rate but is greatly accelerated by hyperglycaemia. In addition to glucose, other dicarbonyl sugars, such as methyl-glyoxal, which forms spontaneously from triose-phosphates, can also modify proteins and subsequently contribute to the formation of AGEs [95,96]. Both AGEs and methyl-glyoxal adducts have been demonstrated to be enhanced in the plasma of diabetic patients [97–100]. At least some AGEs are cleared by the kidney. AGEs have been shown to accumulate in skin, arteries, kidney and blood of diabetic patients, especially in those with renal failure. AGE-adducts can also form on lipids and lipoproteins [101] and increase lipoprotein oxidisability and atherogenicity [102]. AGEs have been demonstrated in atherosclerotic lesions, predominantly at similar locations as epitopes present on oxidised lipoproteins [103,104].

Molecules bearing AGEs acquire new properties. AGE-adducts are oxidants; in addition, they interact with and cross-link basement membrane components, thus impairing proper functioning of the basement membrane [105] and stimulating interaction of mononuclear cells with the modified tissue [106,107]. The introduction of AGEs into the extracellular matrix can interfere with endothelial functioning at different levels. First, AGEs inhibit a normal network formation by type IV collagen [91]. Indeed, infusion of AGE-albumin was shown to interact with the glomerular basement membrane and to induce glomerular sclerosis and albuminuria in rats [105]. Second, they decrease heparan sulphate proteoglycan binding by vitronectin and laminin [91]. Third, they can quench nitric oxide during its passage from the endothelial cell to smooth muscle cells, with loss of its vasodilating and antiproliferative actions [108]. In addition to their direct interaction with matrix proteins, AGEs bind to specific cellular receptors, by which they activate endothelial cells [94,109–111], monocytes [107] and mesangial cells [112]. Yan et al. [109] have suggested that the generation of reactive oxygen species plays a crucial role in endothelial activation by AGEs and causes the activation of NF-kB. The binding of AGEs to their cellular receptor has been shown, in macrophages, to induce the synthesis of interleukin-1, tumour necrosis factor-α, and insulin-like growth factor-1 [91,113], and, in endothelial cells, to increase permeability [94,114] and to induce tissue factor [114], vascular cell adhesion molecule-1 [115], heme oxygenase [109] and interleukin-6 expression [91]. In mice, AGEs also increased glomerular α1(IV)collagen, laminin B1 and TGFβ1 mRNA [112]. AGEs on erythrocytes obtained from diabetic patients induced an increase in endothelial permeability, which could be prevented by anti-AGE-receptor antibodies [116]. Finally, AGE-induced TNFα formation may not only activate endothelial cells and thus affect their antithrombotic properties and the margination and extravasation of leukocytes, but also induce insulin resistance (see Section 6.7).

A new aspect in the cellular activation of endothelial cells by AGEs is the possible formation of intracellular AGEs. Giardini et al. [117] showed that high concentrations of glucose give rise to the formation of glycated basic fibroblast growth factor intracellularly and as such reduces cell proliferation. This observation seems, however, not in par with the finding that AGEs can enhance angiogenesis [118].

Thus, many aspects of vascular dysfunction observed in diabetes are potentially related to the effects of AGEs. The importance of AGEs is further supported by the finding that inhibition of AGE formation by aminoguanidine is associated, in experimental models, with inhibition of the development of diabetic retinopathy, nephropathy, and
large artery stiffening. Clinical trials to assess these effects in humans are under way.

Taken together, the studies reviewed above suggest that AGEs in a proper context can induce or enhance activation of endothelial cells. It still remains to be elucidated what additional factors provide this context, and whether and when this context occurs in diabetic patients. Furthermore, it is unresolved at what stage of the disease AGEs come into play. In IDDM, there is some evidence that high tissue levels of AGEs are associated with and may precede the occurrence of microangiopathy [119]. Data on NIDDM and patients with impaired glucose tolerance are very limited. Assays for AGEs are not widely available, which has hampered a precise assessment of the strength of the relationship between AGEs and the development or presence of diabetic complications. In addition, the biochemical consequences predicted to follow from the presence of AGEs have yet to be conclusively demonstrated in human diabetes.

6.5. Oxidative stress

It is widely thought that the diabetic state is accompanied by increased oxidative stress and that this may be a major pathway in the pathogenesis of diabetic angiopathy [120]. Reactive oxygen species may modify endothelial function by a variety of mechanisms. These include direct effects on the endothelium such as peroxidation of membrane lipids, activation of transcription factors (e.g., NF-kB) leading to the upregulation of adhesion molecules to platelets and leucocytes, and interference with the availability of nitric oxide; and indirect effects such as increasing the oxidation of low-density lipoprotein, the formation of AGEs, and the activation of platelets and monocytes.

Hyperglycaemic pseudohypoxia, glucose auto-oxidation and AGE formation may be important determinants of increased oxidative stress. In addition, hyperglycaemia may impair endothelial free radical scavenging by reducing the activity of the pentose phosphate pathway and thus decreasing the availability of NADPH to the glutathione redox cycle [64]. Moreover, endothelial cells, at least in culture, are very sensitive to reactive oxygen species and lipid peroxides. On the other hand, endothelial cells can respond to high glucose levels by increasing the expression of antioxidant enzymes such as CuZn-superoxide-dismutase, catalase and glutathione peroxidase [121].

It is not known whether oxidative stress causes endothelial dysfunction in human diabetes. It has been difficult to assess the presence of increased oxidative stress in vivo, mainly because of questionable specificity and reproducibility of the methods used. For example, data on lipid peroxides in plasma of diabetic patients using the assay of thiobarbituric reactive substances may need re-assessment, because this assay also recognises glylated proteins. Nevertheless, a recent study, using a specific assay for lipid hydroperoxides, supported the concept of increased oxidative stress in NIDDM [122]. In addition, there is some evidence that oxidative stress may be linked to endothelial dysfunction in NIDDM [42].

6.6. Activation of the coagulation cascade

Diabetes in humans is associated with activation of the coagulation cascade at multiple levels, with increases in factor VII activation and thrombin and fibrin formation and a decreased activity of antithrombin III [123]. Data on the activity of the important anticoagulant defense, the protein S–protein C system are conflicting: both decreased and increased activity have been reported [5,123]. There is some evidence of increased anticoagulant defense activity: the anticoagulant response to activated protein C may be enhanced [124] and the secretion of tissue factor pathway inhibitor, an endothelium-derived protein, may be increased [125,126]. The activation of the coagulation cascade is accentuated in patients with micro- and macroalbuminuria (i.e., in those with relatively severe endothelial dysfunction) [4,5,123,125,127,128].

Hyperglycaemia is thought to activate the coagulation cascade by various mechanisms, such as non-enzymatic glycation (which may decrease antithrombin III activity), AGE formation (which may increase tissue factor activity), impairment of heparan sulphate synthesis [129] (which decreases antithrombin III co-factor activity), and increased oxidative stress [130]. It is likely that, later in the course of diabetes, loss of endothelial anticoagulant properties further enhances activation of coagulation [5].

There is no direct evidence that activation of the coagulation cascade causes endothelial injury in human diabetes. Nevertheless, thrombin [9] and fibrin fragments [131] are strong stimulators of endothelial cells. Therefore, repeated activation of coagulation may cause overstimulation of endothelial cells and endothelial dysfunction. This possibility deserves further study.

6.7. Tumour necrosis factor-α (TNF-α)

Recent provocative studies have indicated that increased adipose tissue and muscle expression of TNF-α in human obesity may induce insulin resistance [132,133] through its ability to impair intracellular signalling from the insulin receptor by inhibition of insulin-stimulated autophosphorylation of the insulin receptor and phosphorylation of insulin receptor substrate-1 [134,135].

TNF-α can induce the synthesis of other powerful cytokines, such as interleukin-6, and, alone or in concert with other cytokines, can alter endothelial function. It is not yet known to what extent chronic, moderate elevations of cytokine levels induce endothelial dysfunction in diabetes; this is an important area for further study. The link between insulin resistance and TNF-α provides the basis for a novel hypothesis to explain insulin-resistance-associated vascular disease: could it be caused by cytokine-in-
duced inflammatory changes in the vascular wall that increase atherogenesis and thrombogenesis? Of note, the fact that TNF-α activity is increased in insulin-resistant states potentially provides an explanation for the finding that NIDDM [136] and obesity [137] are associated with slight increases in the levels of acute-phase proteins, such as C-reactive protein and fibrinogen, which in turn are markers of an adverse cardiovascular prognosis under various circumstances [14,138]

6.8. Insulin

Endothelial cells have insulin receptors, but the effects of hyperinsulinaemia on endothelial function have not yet been extensively studied.

In vitro, insulin can increase endothelial production of endothelin [139], a potent vasoconstrictor and mitogen. Insulin can also increase endothelin levels in obese humans [140]. On the other hand, insulin enhances nitric oxide synthesis, an action that is impaired in insulin-resistant subjects [49–51]. Polderman et al. have recently investigated the effects of insulin infusion on endothelium-derived vasoactive mediators in healthy men and women with normal insulin sensitivity [141]. They observed an increase in the plasma levels of markers of nitric oxide synthesis in women but not in men, and a decrease in the plasma levels of endothelin in women but no change in men. They proposed that insulin has a dual effect on endothelin synthesis by the endothelium: a direct stimulatory effect and an indirect inhibitory effect mediated by an increase in nitric oxide synthesis. They further speculated that, in healthy women, the latter effect is predominant; in insulin-resistant subjects, insulin’s effect on nitric oxide synthesis is impaired, which might explain insulin-induced increases in plasma endothelin levels in obese women [140].

Insulin and insulin precursors may increase plasma levels of PAI-1, although the source of PAI-1 is controversial [16,47,142]. In any case, high PAI-1 levels inhibit fibrinolysis and facilitate the persistence of fibrin, which may damage the endothelium.

Whether insulin has other atherogenic effects is controversial, mainly because it is not clear whether such effects occur at physiological concentrations [143]. For example, insulin can stimulate vascular smooth muscle cell proliferation [47,144]. Insulin may also increase vascular permeability to macromolecules, possibly through changes in endothelial morphology and para-endothelial permeability [145]. Moreover, insulin may indirectly increase the risk of atherosclerotic disease through its associations with hypertension and dyslipidaemia (high triglyceride and low HDL-cholesterol levels). There is no consensus on whether or not hypertension and dyslipidaemia cause atherosclerosis primarily through endothelial injury and dysfunction. This discussion, which is not specific to diabetes, is beyond the scope of this review [40,144,146–151]. Nevertheless, the scant clinical data available in diabetes do not support a role for hypertension and dyslipidaemia very early in the pathogenesis of endothelial dysfunction in diabetic angiopathy. For example, in a prospective study in NIDDM, blood pressure and lipid levels were not strongly related to vWF levels or to the development of increases in urinary albumin excretion [23]. Microalbuminuria may be associated with a slightly increased blood pressure [4], but whether this is the cause of microalbuminuria or the consequence of the processes underlying the development of microalbuminuria is not clear.

Finally, provocative in vivo data indicate that C-peptide may have effects on vascular tone and permeability [152], although the molecular basis for these effects has not been elucidated. These findings nevertheless merit further investigation in view of the fact that diabetic patients usually have very abnormal blood levels of C-peptide (low in IDDM and high in NIDDM).

7. Summary

The endothelium is an important locus of control of vascular and renal functions. Reasonable but not perfect methods exist for assessing endothelial function in vivo in humans. This paper examines the contribution of dysfunction of the vascular endothelium to the pathogenesis of diabetic micro- and macroangiopathy from the clinical perspective.

Available data indicate that endothelial dysfunction in diabetes complicated by micro- or macroalbuminuria is generalised, in that it affects many aspects of endothelial function. The close linkage between microalbuminuria and endothelial dysfunction in diabetes is an attractive explanation for the fact that microalbuminuria is a risk marker for atherosclerotic cardiovascular disease.

Although endothelial dysfunction precedes the occurrence of even early diabetic microangiopathy, it is not clear whether endothelial dysfunction is a feature of the diabetic state per se or whether additional factors are required to induce endothelial dysfunction given the presence of diabetes. Of note, improved glycaemic control for up to 3 years does not clearly ameliorate endothelial dysfunction, as estimated by increased urinary albumin excretion and elevated von Willebrand factor plasma levels [5,153,154].

Various biochemical mechanisms have been proposed to be at the heart of the pathogenesis of diabetic angiopathy. This paper reviews the relationships between endothelial dysfunction and a number of leading contenders: hyperglycaemic pseudohypoxia, activation of protein kinase C, increased expression of transforming growth factor-β and vascular endothelial growth factor, non-enzymatic glycation, oxidative stress, activation of the coagulation cascade, increased expression of tumour necrosis factor-α, and high levels of insulin and insulin precursor molecules.
The relevance of these biochemical mechanisms for the development of endothelial dysfunction in diabetes in man has not yet been extensively investigated.

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