Vascular complications in diabetes mellitus: the role of endothelial dysfunction

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ABSTRACT

The endothelium is a complex organ with a multitude of properties essential for control of vascular functions. Dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of diabetic micro- and macro-angiopathy. Endothelial dysfunction in Type I and II diabetes complicated by micro- or macro-albuminuria is generalized in that it affects many aspects of endothelial function and occurs not only in the kidney. The close linkage between microalbuminuria and endothelial dysfunction in diabetes is an attractive explanation for the fact that microalbuminuria is a risk marker for atherothrombosis. In Type I diabetes, endothelial dysfunction precedes and may cause diabetic microangiopathy, but it is not clear whether endothelial dysfunction is a feature of the diabetic state itself. In Type II diabetes, endothelial function is impaired from the onset of the disease and is strongly related to adverse outcomes. It is not clear whether impaired endothelial function is caused by hyperglycaemia or by other factors. Impaired endothelial function is closely associated with and may contribute to insulin resistance regardless of the presence of diabetes. Endothelial dysfunction in diabetes originates from three main sources. Hyperglycaemia and its immediate biochemical sequelae directly alter endothelial function or influence endothelial cell functioning indirectly by the synthesis of growth factors, cytokines and vasoactive agents in other cells. Finally, the components of the metabolic syndrome can impair endothelial function.

INTRODUCTION

Diabetes mellitus is a common metabolic disease worldwide affecting approx. 150 million people in 2000, which is predicted to rise to 220 million in 2010 [1]. Diabetes and its associated complications have become a public health problem of considerable magnitude. Cardiovascular disease causes most of the excess morbidity and mortality in diabetes mellitus. Adults with diabetes are at a 2- to 4-fold increased risk of cardiovascular events relative to those without diabetes [2]. Cardiovascular disease accounts for up to 80% of premature excess mortality in diabetic
patients [3]. Because of the huge premature morbidity and mortality associated with this disease, prevention of complications is a key issue.

Vascular complications can be caused by micro- and macroangiopathy. Retinal and renal microangiopathy cause diabetic retinopathy and nephropathy and microangiopathy of the vasa nervorum is important in the pathogenesis of neuropathy. Macroangiopathy in diabetes consists mainly of an accelerated form of atherosclerosis and affects the coronary, carotid and peripheral arteries, thus increasing the risk of myocardial infarction, stroke and diabetic foot disease [4–7].

Large clinical trials in both Type I and Type II diabetes have demonstrated that hyperglycaemia plays an important role in the pathogenesis of microvascular complications [8,9]. Although diabetic patients with the most severe hyperglycaemia have the highest risk of microangiopathy, hyperglycaemia, however, is a necessary, but not a sufficient, cause of clinically important microangiopathy. Hypertension, smoking, hypercholesterolaemia, dyslipidaemia, obesity and hyperhomocysteinaemia are additional major causes of microangiopathy. Risk of macroangiopathy does not appear to be strongly related to hyperglycaemia, but is related to general risk factors for atherothrombosis, such as age, smoking, hypertension, hypercholesterolaemia, dyslipidaemia, obesity and hyperhomocysteinaemia. The cardiovascular risk factors hypertension, dyslipidaemia, obesity, insulin resistance, hyperinsulinaemia and impaired fibrinolysis cluster in the metabolic syndrome. All of the above-mentioned factors create a state of constant and progressive damage to the vascular wall, manifested by a low-grade inflammatory process and endothelial dysfunction [10].

Dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of micro- and macroangiopathy [11,12] and endothelial function has gained increasing attention in the study of vascular disease. This review will explore how endothelial dysfunction in diabetes relates to the pattern of disease occurrence described above and what current biochemical mechanisms have been proposed as an explanation for the development of endothelial dysfunction in diabetes.

ENDOTHELIAL FUNCTION AND DYSFUNCTION

The endothelium is the biological active inner layer of the blood vessels, which serves as an important locus of control of vascular and thus organ functions [13]. The endothelium actively regulates vascular tone and permeability, the balance between coagulation and fibrinolysis, the composition of the subendothelial matrix, the adhesion and extravasation of leucocytes, and inflammatory activity in the vessel wall. It also affects the functions of other cell types, such as vascular smooth muscle cells, platelets, leucocytes, retinal pericytes, renal mesangial cells and large artery macrophages. On the other hand, these cells can affect endothelial cells. To carry out its above-mentioned functions, the endothelium produces components of the extracellular matrix such as collagen and a variety of regulatory mediators, including NO (nitric oxide), prostanoids, ET-1 (endothelin-1), Ang II (angiotensin II), t-PA (tissue-type plasminogen activator), PAI-1 (plasminogen activator inhibitor-1), vWF (von Willebrand factor), adhesion molecules and cytokines. The production of these moieties is responsive to various stimuli [14]. Normally, the endothelium actively decreases vascular tone, limits leucocyte adhesion and thus inflammatory activity in the vessel wall, maintains vascular permeability to nutrients, hormones, other macromolecules and leucocytes, inhibits platelet adhesion and aggregation by producing prostacyclin and NO, limits activation of the coagulation cascade by the thrombomodulin/protein C, heparan sulphate/antithrombin and tissue factor/tissue factor pathway inhibitor interactions, and regulates fibrinolysis by producing t-PA and its inhibitor PAI-1.

Among important molecules synthesized by endothelial cells is NO, which is a particularly important endothelium-derived mediator, because of its vasodilator, anti-platelet, anti-proliferative, permeability-decreasing and anti-inflammatory properties [15]. NO inhibits leucocyte adhesion and rolling as well as cytokine-induced expression of VCAM-1 (vascular cell adhesion molecule-1) and MCP-1 (monocyte chemoattractant protein-1) [16], effects that are at least in part attributable to inhibition of the transcription factor NF-κB (nuclear factor κB) [17].

Injury to the endothelium can cause endothelial dysfunction. 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. 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). Endothelial activation designates one specific set of dysfunctions characterized by increased interactions with blood leucocytes in which adhesion molecules and chemoattractants such as MCP-1 and IL (interleukin)-8 are essential.

Endothelial dysfunction is thought to play an important role not only in the initiation of atherosclerosis, but also in its progression and clinical sequelae. Risk factors such as hypercholesterolaemia, dyslipidaemia, smoking and diabetes initiate atherosclerosis through endothelial activation and therefore endothelial dysfunction. Therefore endothelial activation can be conceptualized as a transducer of atherogenic risk factors. These
Table 1  Proposed estimates of endothelial dysfunction in humans

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Interpretation of altered endothelial function</th>
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<tr>
<td>Impaired endothelium-dependent vasodilation</td>
<td>Decreased production of vasodilators and/or increased production of vasoconstrictors</td>
</tr>
<tr>
<td>Increased transcapillary escape rate of intravenously injected radiolabelled albumin, microalbuminuria and sCD146</td>
<td>Increased permeability to macromolecules</td>
</tr>
<tr>
<td>↑Endothelin</td>
<td>Increased production of vasoconstrictors</td>
</tr>
<tr>
<td>↑vWF</td>
<td>Increased prothrombotic and procoagulant activity</td>
</tr>
<tr>
<td>↑Thrombomodulin</td>
<td>Decreased anticoagulant activity</td>
</tr>
<tr>
<td>↑PA and PAI-1</td>
<td>Decreased plasmatic activity</td>
</tr>
<tr>
<td>↑sE-selectin and sVCAM-1</td>
<td>Increased adhesion and permeability to leucocytes</td>
</tr>
<tr>
<td>↑sICAM-1</td>
<td>Inflammatory activation</td>
</tr>
<tr>
<td>↑Cellular fibronectin and Type IV collagen fragments</td>
<td>Altered extracellular matrix synthesis</td>
</tr>
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atherogenic risk factors have in common that endothelial NO availability is decreased, whether through decreased production or through increased degradation. For example, high LDL (low-density lipoprotein)-cholesterol increases the production of ROS (reactive oxygen species) and thus NO scavenging, but also increases the interaction between NOS (NO synthase) and caveolin-1, which decreases NO production. Another important example is Ang II, which increases vascular NAD(P)H oxidases, superoxide production and NO scavenging, and thus decreases NO availability. These processes will lead to endothelial activation manifested by increased expression of adhesion molecules [16].

MEASUREMENT OF ENDOTHELIAL DYSFUNCTION

Endothelial function cannot be measured directly in humans. Estimates of different types of endothelial dysfunction may be obtained indirectly by measuring endothelium-dependent vasodilation, plasma levels of endothelium-derived regulatory proteins and, possibly, microalbuminuria. Some other vascular properties, such as arterial stiffness and intima/media thickness in the carotid artery, are probably in part endothelium-dependent, but it is not known to what extent. It is not entirely certain whether the measurements of endothelial function, as listed in Table 1, are valid estimates of endothelial dysfunction. On the one hand, many cross-sectional studies have shown impaired endothelium-dependent vasodilation and high levels of endothelium-derived regulatory proteins in patients with diseases that involve injury to the endothelium, such as atherothrombosis, pre-eclampsia and vasculitis, as well as in individuals with risk factors for atherothrombosis [18–20]. Moreover, prospective studies have shown that individuals with impaired endothelium-dependent vasodilation, high levels of endothelium-derived regulatory proteins or microalbuminuria have an adverse cardiovascular prognosis [21–23]. On the other hand, some questions can be raised about the validity of the tests listed in Table 1. First, tests intended to estimate NO-mediated endothelium-dependent vasodilation in part measure the effects of other endothelial vasodilators such as prostacyclin and EDHF (endothelium-derived hyperpolarizing factor), and may partly be confounded by impaired vascular smooth muscle function. Secondly, the concept that high plasma levels of endothelium-derived mediators reflect endothelial dysfunction in clinically relevant arteries (such as the coronary and carotid) requires that (i) other cell types are not an important source; (ii) synthesis is more important than clearance, unless the latter should be endothelium dependent; and (iii) endothelial function in the microvasculature parallels that in large arteries, the latter because of the fact that microvascular endothelium, with its very large surface area and synthetic capacity, is the most important determinant of plasma levels of endothelium-derived mediators. Information on the validity of these assumptions is as yet scarce. In some cases, the assumptions are clearly invalid. For example, PAI-1 can be produced not only by endothelial cells, but also by hepatocytes, adipocytes and vascular smooth muscle cells [24]. Thirdly, 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 [25] and by haemodynamic forces [26]. In this regard, novel data with soluble CD146, a member of the immunoglobulin superfamily, which is involved in the control of cell–cell cohesion and thus endothelial permeability [27], may provide additional information about the involvement of the endothelium in vascular permeability. Thus reasonable, but not perfect, estimates exist for assessing endothelial function in vivo in humans.

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ENDOTHELIAL DYSFUNCTION IN DIABETES

General remarks
A considerable body of evidence in humans indicates that endothelial dysfunction is closely associated with the development of diabetic retinopathy, nephropathy and atherosclerosis in both Type I and Type II diabetes [12,28]. However, the literature on endothelial function in diabetes is complex in part because endothelial function can be measured in many ways and in many vascular beds. In addition, endothelial function in diabetes has been investigated to answer several distinct questions, such as (i) is the occurrence of microalbuminuria accompanied by severe endothelial dysfunction; (ii) is hyperglycaemia a sufficient cause of at least some types of endothelial dysfunction; (iii) what is the significance of the finding that insulin resistance is associated with endothelial dysfunction and (iv) what are the mechanisms that cause diabetes-associated endothelial dysfunction?

Generalized endothelial dysfunction, chronic low-grade inflammation, microalbuminuria and atherothrombosis
Although a majority of diabetic patients will develop vascular complications, a substantial fraction will never develop severe vascular complications. Thus, within the group of diabetic patients, a subgroup exists with a relatively normal compared with a very high risk of cardiovascular complications. In both types of diabetes, patients with advanced nephropathy, i.e. macroalbuminuria, have very high risk of developing severe complications [29–31]. The pattern of increased risk for vascular complications can be observed even in early nephropathy, i.e. microalbuminuria, in Type II diabetes [32] and also in non-diabetic subjects [33], which has raised the question of what common mechanisms may be at work. Because micro- and macro-albuminuria are often associated with classic risk factors for microangiopathy and atherothrombosis, notably poor glycaemic control, hypertension, obesity, dyslipidaemia and smoking [34], an obvious possibility is that such risk factors cause both (micro)albuminuria and atherothrombosis and thus explain their association. Many studies have investigated this and have concluded that common risk factors explain at most a small part of the association between (micro)albuminuria and atherothrombosis [32,35,36]. Other mechanisms must therefore be at work, which may include severe generalized endothelial dysfunction [37] and chronic low-grade inflammation [38].

Indeed, in both types of diabetes, micro- and macroalbuminuria are accompanied by a variety of markers of endothelial dysfunction. For example, we recently demonstrated [22], in a study of 328 patients with Type II diabetes who were followed for a mean of 9 years, that albumin excretion rate was significantly and independently correlated with levels of plasma vWF and soluble E-selectin. We concluded that, in Type II diabetes, increased urinary albumin excretion and endothelial dysfunction develop in parallel, progress with time, and are strongly and independently associated with risk of death. Prospective studies using markers such as plasma vWF have shown that high vWF concentrations are associated with an increased risk of developing microalbuminuria, an increased progression of microalbuminuria, the occurrence of diabetic retinopathy and neuropathy and an increased risk of cardiovascular events and death [39–42]. Endothelial dysfunction in Type I and II diabetes complicated by micro- or macro-albuminuria is generalized in that it affects many aspects of endothelial function and occurs both in the kidney and elsewhere. Such data, together with more limited data showing that microalbuminuria is also associated with endothelial dysfunction in the absence of diabetes [37,43], have led to the concept that microalbuminuria itself is a marker of generalized renal and extrarenal endothelial dysfunction.

It is less clear how endothelial dysfunction would cause (micro)albuminuria. Theoretically, endothelial dysfunction could contribute to the pathogenesis of albuminuria both directly, by causing increased glomerular pressure and the synthesis of a leaky glomerular basement membrane, and indirectly, by influencing glomerular mesangial and epithelial cell function in a paracrine fashion. Importantly, the molecular pathways by which endothelial dysfunction causes (micro)albuminuria have yet to be worked out.

Chronic low-grade inflammation is another candidate to explain the association between (micro)albuminuria and extrarenal complications [22,44,45]. Indeed, regardless of the presence of diabetes, chronic low-grade inflammation is associated with the occurrence and progression of (micro)albuminuria [44] and with risk of atherothrombotic disease [46]. In addition, chronic low-grade inflammation can be both cause and consequence of endothelial dysfunction, and the two appear tightly linked [22,44,45]. Nevertheless, recent data indicate that the association between (micro)albuminuria and atherothrombosis cannot be explained entirely by markers of endothelial dysfunction and chronic inflammation [22,32]. One possibility is that such markers do not fully capture the processes they are meant to reflect; an alternative or additional explanation is that there are other pathways that link (micro)albuminuria to extrarenal complications, such as autonomic neuropathy [47,48] or prothrombotic mechanisms (Figure 1).

At what stage of diabetes does endothelial dysfunction become manifest?

Type I diabetes
In Type I diabetes, patients who have had diabetes for more than 5–10 years and who have normal urinary
albumin excretion compared with non-diabetic individuals are characterized by subtle increases in blood pressure and large-artery stiffness and by autonomic dysfunction, and some or perhaps most have impaired endothelial function and increased low-grade inflammation. All these abnormalities are worse in the microalbuminuric stage [26,45,49]. It is not clear how they interact or whether impaired endothelial function is a common antecedent or a consequence, but these data illustrate that cardiovascular function, including endothelial function, does become impaired before the onset of microalbuminuria, at least when the latter is defined as \( \geq 30 \text{ mg/24 h} \). The possibility remains, however, that patients with ‘normal’ urinary albumin excretion who show such cardiovascular abnormalities are those in whom urinary albumin excretion is in fact increased albeit within the normal range as conventionally defined, i.e. the (arbitrary) cut-off of 30 mg/24 h may be too high. This has a parallel in Type II diabetes and in non-diabetic individuals in whom any increase in microalbuminuria appears associated with increased risk of atherothrombosis [32,36].

Endothelial dysfunction thus occurs before microalbuminuria sets in. However, is endothelial dysfunction a feature of Type I diabetes and is moderate hyperglycaemia sufficient to impair endothelial function? On the one hand, plasma vWF levels are not increased in patients with early uncomplicated and reasonably well-regulated Type I diabetes, suggesting normal endothelial function. On the other hand, it has been argued that NO-mediated endothelium-dependent vasodilation is impaired in short-term uncomplicated diabetes and that hyperglycaemia, in the absence of other factors, acutely impairs endothelium-dependent vasodilation in non-diabetic individuals [50,51]. Several findings are, however, in conflict with these concepts [52–55]. First, studies that carefully stratified patients according to the absence or presence of a normal urinary albumin excretion rate have concluded that, in reasonably well-controlled Type I diabetes, endothelium-dependent and -independent vasodilation of resistance and conduit arteries are neither impaired nor enhanced [52–55]. Secondly, early uncomplicated Type I diabetes is accompanied by dilation, not constriction, of small and large blood vessels and an increase in microvascular blood flow, both in humans and in animal models [54]. Microvascular dilation may cause capillary hypertension and, according to the so-called haemodynamic hypothesis of the pathogenesis of microangiopathy, capillary hypertension will, in time, damage the microvascular endothelium and thus set the stage for more advanced stages of microangiopathy, such as microalbuminuria. It is at this stage that a general impairment of endothelial function, including impaired endothelium-dependent vasodilation, can usually be clearly observed, although endothelial function may sometimes be normal even among microalbuminuric individuals [56]. Notably, the mediators responsible for the vasodilation typical of early Type I diabetes remain to be identified and it is not known whether the endothelium is involved. Taken together, these data suggest that impaired endothelium-dependent vasodilation may occur early in a subset of Type I diabetic patients apparently dependent on other environmental or genetic factors such as the Ang II type I receptor gene polymorphism [57]. In other words, the diabetic state predisposes to the development of endothelial dysfunction, but in and of itself is not a sufficient cause. Other factors, genetic or environmental, are likely to play a role in determining who among Type I diabetic patients go on to develop aggressive angiopathy and who do not.

**Type II diabetes**

Endothelial dysfunction indicated by impaired endothelium-dependent vasodilation and increased plasma concentrations of markers of endothelial function is common in early and otherwise uncomplicated Type II diabetes. For example, vWF and soluble VCAM-1 levels are increased and have been found to be associated with an increased risk of cardiovascular mortality and development and progression of microalbuminuria [22,44]. Impairment of endothelial function may be especially severe among diabetic women [58].

It is not clear to what extent this endothelial dysfunction is caused by hyperglycaemia. Type II diabetes typically occurs in the context of a cluster of cardiovascular risk factors, notably obesity, hypertension, high triacylglycerol (triglyceride) levels, low HDL (high-density lipoprotein)-cholesterol levels, abnormal LDL composition, hyperinsulinaemia, insulin resistance and chronic low-grade inflammation, all of which may impair endothelial function. Endothelial dysfunction in Type II diabetes appears to be independent of that induced by obesity [59], but the role of the other variables (compared with hyperglycaemia) remains to be established. An important contender is increased inflammatory activity. Endothelial dysfunction, inflammation and urinary albumin excretion in Type II diabetes are progressive and closely interrelated [22]. Nevertheless, as in Type I
disposal both when the microvascular endothelium is
endothelial dysfunction and impaired capillary recruit-
ment [65–68]. Taken together, these data suggest that
esterified fatty acids) and an impaired NO-dependent
to insulin resistance, namely
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ifiers closely associated with insulin resistance, namely
TNF-α (tumour necrosis factor-α), NEFAs (non-
esterified fatty acids) and an impaired NO-dependent
action [65–68]. Taken together, these data suggest that
endothelial dysfunction and impaired capillary recruit-
ment can cause insulin resistance with respect to glucose
disposal both when the microvascular endothelium is
otherwise healthy but cannot react properly to insulin
(‘endothelial insulin resistance’) and when the microvas-
cular endothelium is injured through other mechanisms,
such as age-related capillary drop-out (‘rarefaction’, i.e.
reduced capillary density per volume of tissue). Speci-
ically, decreased capillary density and impaired capillary
recruitment may decrease insulin-mediated glucose dis-
posal by increasing the diffusion distance of glucose
and insulin to glucose-metabolizing tissues, by impairing
transendothelial insulin transport, if this should be
surface-area-dependent, and by impairing recruitment of
previously underperfused muscle tissue.

Decreased capillary density and impaired capillary
recruitment may also play a role in the development of
atherogenic changes in lipoprotein concentrations
through impaired action of endothelium-bound LPL
(lipoprotein lipase). LPL is the rate-limiting enzyme for
triacylglycerol utilization and its physiological site of
action is the capillary endothelial surface [72]. A reduced
capillary endothelial surface area may in turn result in
reduced access of triacylglycerol-rich lipoprotein particles
to LPL. Such a mechanism could explain why dyslipi-
daemia of the metabolic syndrome is confined to triacyl-
glycerols and HDL-cholesterol.

How can endothelial dysfunction impair insulin-
induced glucose disposal? First, insulin is a vasoactive
hormone. Insulin increases muscle blood flow in a time-
and concentration-dependent fashion through a mech-
anism that involves binding to the insulin receptor on
the endothelial cell membrane. Nevertheless, insulin-
induced increases in glucose uptake and total blood flow
have different concentration–effect curves as well as
time kinetics. Therefore it is unlikely that a simple
insulin-induced increase in total blood flow can increase
glucose disposal. Secondly, however, in a process termed
capillary recruitment, insulin can redirect blood flow
in skeletal muscle from non-nutritive capillaries (those
that are not coupled to muscle cells) to nutritive capil-
laries (those that are) and thus increase glucose disposal
even without increasing total blood flow [62–64]. In
this way, physical integrity and normal function of the
arteriolar and capillary endothelium are prerequisites
for normal metabolic insulin action. Indeed, insulin’s
vasodilator actions have been shown to be impaired in
classic insulin-resistant states, notably Type II diabetes,
obesity and hypertension, and to be decreased by medi-
ators closely associated with insulin resistance, namely
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ENDOTHELIAL DYSFUNCTION, INSULIN
RESISTANCE AND THE METABOLIC
SYNDROME

Insulin resistance usually precedes the development of
Type II diabetes and is often accompanied by a cluster
of other risk factors (see above). The mechanisms under-
lying this clustering are still unclear, but all elements of the
cluster share two important pathophysiological features,
namely insulin resistance and endothelial dysfunction.
A widely accepted theory states that insulin resistance
is the primary abnormality that gives rise to Type II
diabetes, hypertension and dyslipidaemia, and that
endothelial dysfunction merely represents the impact
of hyperglycaemia and other features of the metabolic
syndrome. An alternative concept is that endothelial
dysfunction is at the heart of the metabolic syndrome.
According to this concept, the endothelial dysfunction
in large arteries that is an early and prominent event in
atherothrombotic disease is paralleled by endothelial dys-
fuction in resistance vessels and metabolically impor-
tant capillary beds that contributes to the development
of the metabolic syndrome [61].

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The molecular pathways through which insulin
increases NO synthesis, endothelium-dependent vaso-
dilation and capillary recruitment have not yet been
fully elucidated, or how TNF-α, NEFAs and perhaps
hyperinsulinaemia itself impair these actions of insulin.
First, insulin can act on insulin receptors on endo-
thelial cells to produce NO, but also ET-1, a potent
vasoconstrictor. Endothelial insulin resistance can thus
be conceptualized as a shift in the balance between vaso-
dilators and vasoconstrictors produced by insulin, with
vasodilation as the normal response and impaired vaso-
dilation or even net vasoconstriction as abnormal re-
ponses [73]. Secondly, although insulin’s endothelial
actions have been shown to occur in cell culture and
in isolated vessels, this does not exclude that, in vivo,
insulin may act at insulin receptors on vascular smooth
cells to cause vasodilation and (or) on skeletal muscle
to activate glucose metabolism to produce a metabolite (e.g.,
adenosine) that then acts on local endothelial and (or)
smooth muscle cells. Thirdly, regardless of the presence
of diabetes, chronic low-grade inflammation is closely
associated with, and may link, endothelial dysfunction
Vascular complications in diabetes mellitus: the role of endothelial dysfunction

MECHANISMS UNDERLYING ENDOTHELIAL DYSFUNCTION IN DIABETES

General remarks
Endothelial dysfunction in diabetes originates from three main sources [77–80]. First, hyperglycaemia and its immediate biochemical sequelae directly alter endothelial function [77]. Glucose transport into endothelial and vascular smooth muscle cells occurs by facilitated diffusion and is thus insulin-independent. Glucose transport is autoregulated by glucose in smooth muscle cells, but not in endothelial cells, in which an increase in blood glucose concentration will thus increase the intracellular accumulation of glucose and its metabolites. Thus 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 von Willebrand factor (vWF) and tissue factor, and show decreased proliferation, migration and fibrinolytic potential, and increased apoptosis [81–86]. Secondly, high glucose influences endothelial cell functioning indirectly by the synthesis of growth factor and vasoactive agents in other cells [87]. Thirdly, the components of the metabolic syndrome can affect endothelial function [88].

Hyperglycaemia and its immediate biochemical sequelae
Various mechanisms have been proposed to explain how hyperglycaemia directly causes diabetic vascular complications. An increase in intracellular glucose will lead to an increase in the flux of glucose to sorbitol via the polyol pathway, an increase in glucosamine-6-phosphate via the hexosamine pathway, and the activation of protein kinase C (PKC) (protein kinase C) via de novo synthesis of diacylglycerol (DAG). In addition, glucose and glucose-derived dicarbonyl compounds react non-enzymatically with the basic amino acids lysine and arginine in proteins to form AGEs (advanced glycosylation end-products) both extra- and intra-cellularly. These different pathways are interrelated and potentiate each other. Figure 3 shows how, intracellularly, these four biochemical mechanisms may all be the consequence of hyperglycaemia-induced overproduction of ROS in mitochondria.

The sorbitol pathway
In most cells, excess glucose can be metabolized to sorbitol and fructose by aldose reductase and sorbitol dehydrogenase, which is accompanied by increased oxidation of NADPH to NADP⁺ and increased reduction of NAD⁺ to NADH [89,90]. This pathway may impair endothelial function through three mechanisms. First, increased sorbitol accumulation will increase osmotic stress. Sorbitol accumulation decreases other osmolytes such as myo-inositol and taurine. However, the relatively low expression of aldose reductase in endothelial cells may not be sufficient to cause significant sorbitol accumulation. Secondly, the increase in the cytosolic NADH/NAD⁺ ratio results in a redox imbalance that resembles that which occurs in tissue hypoxia and therefore is termed hyperglycaemic pseudohypoxia [91]. Thirdly, the redox imbalance favours the accumulation of triose phosphates which increases the formation of methylglyoxal and AGEs and enhances oxidative stress, which can be exacerbated by NADPH-deficiency-induced depletion of reduced glutathione.

The full impact of the sorbitol pathway in vascular dysfunction is not completely understood and the role of inhibition of aldose reductase in the prevention and treatment of diabetic complications remains unclear. Aldose reductase activity in endothelial cells of different origin is low and it thus appears unlikely that the improved nerve conduction in diabetic neuropathy observed with aldose reductase inhibitors or myo-inositol supplementation is related to improved endothelial function. In contrast, an excess aldose reductase activity in human retinal endothelial cells can be a mechanism for human diabetic retinopathy [92].

The DAG/PKC pathway
The cellular pathogenic consequences of hyperglycaemia-induced activation of PKC are multiple and include dysregulation of vascular permeability directly or indirectly (the latter through the induction of vascular endothelial growth factor) in smooth muscle cells [93], dysregulation of blood flow by decreasing endothelial NOS activity and (or) increasing ET-1 synthesis [94], basement membrane thickening through TGF-β (transforming growth factor-β)-mediated increased synthesis of type IV collagen and fibronectin, impaired fibrinolysis through increased expression of PAI-1, and increased oxidative stress by the regulation of several NADPH oxidases.
Various mechanisms have been proposed to explain how hyperglycaemia causes diabetic vascular dysfunction. Under normal conditions, glucose is metabolized through the glycolytic pathway. An increase in intracellular glucose will lead to an increase in four pathways: the flux of glucose to sorbitol via the sorbitol pathway, an increase in fructosamine 6-phosphate via the hexosamine pathway, the activation of PKC via de novo synthesis of DAG, and the formation of AGEs. In several cell types, excess glucose can be metabolized in the sorbitol pathway to sorbitol and fructose by aldose reductase (AR) and sorbitol dehydrogenase (SDH). In the hexosamine pathway, fructose 6-phosphate is converted into fructosamine 6-phosphate by the enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT) and, subsequently, into GlcNAc. The mechanism responsible for the activation of PKC by hyperglycaemia is related to de novo synthesis of the PKC activator DAG from a stepwise acylation of glycerol 3-phosphate (GP) and phosphatidic acid (PA). Recent evidence indicates that these four biochemical and metabolic mechanisms are the consequence of a hyperglycaemia-induced overproduction of oxidative stress in the mitochondria. Three of the major biochemical pathways implicated in the pathogenesis of hyperglycaemia-induced vascular damage (the hexosamine pathway, the DAG/PKC pathway and the formation of AGEs) can be inhibited by the lipid-soluble thiamine derivative benfotiamine by activating the pentose phosphate pathway (PPP) enzyme transketolase (TK) [78,117]. In addition to this pathway, hyperglycaemia may lead to increased ROS by activation of NAPDH oxidase, inactivation and reduced expression of the antioxidant enzymes catalase and superoxide dismutase (SOD), or uncoupling of eNOS. Adapted from [77] with permission. © (2001) Nature Publishing Group (http://nature.com/).

Hyperglycaemia-induced activation of PKC occurs through increased levels of the PKC activator DAG which, in cells with a low aldose reductase activity such as endothelial cells, is synthesized de novo from the glycolytic intermediates dihydroxyacetone phosphate and glyceroldehyde-3-phosphate [95]. Interestingly, vitamin E can inhibit PKCβ activity, indicating a link between oxidative stress and PKC activation [96].

The PKC family consists of at least eleven isoforms [97]. In vascular cells, the PKCβII isofom appears preferentially activated [98]. In diabetic animals, an oral PKCβ inhibitor prevented diabetes-induced abnormalities in mRNA expression of TGF-β1, type IV collagen and fibronectin, ameliorated increases in glomerular filtration rate and accelerated glomerular mesangial expansion, and partly corrected urinary albumin excretion [99]. Studies to evaluate the importance of the DAG/PKC pathway in humans are underway.

The hexosamine pathway

The vascular effects of the hexosamine pathway, in which fructose 6-phosphate is converted into glucosamine 6-phosphate by the enzyme glutamine:fructose-6-phosphate amidotransferase, are just beginning to be understood but may be profound [100]. In aortic endothelial cells, hyperglycaemia was shown to inerease levels of hexosamine 6-phosphate and subsequently GlcNAc (N-acetylglucosamine). This, by the addition of GlcNAc to serine and threonine residues, increased O-linked glycosylation of the transcription factor SP-1, which decreased SP-1 phosphorylation and increased SP-1 activity. In turn this can increase transcription of PAI-1 and TGF-β1 [101]. Other proteins, such as PKC and endothelial cell NOS can be modified in a similar way. For example, such a modification of the Akt site of endothelial cell NOS has been shown to decrease enzyme activity [102].
Non-enzymatic glycation

Non-enzymatic glycation of proteins is the condensation reaction of the carbonyl group of sugar aldehydes with the N-terminus of free amino acids of proteins and initially leads to a Schiff’s base, which then undergoes rearrangement to early glycation Amadori-adducts such as fructosamine [103]. Amadori-adducts are relatively stable and only a small fraction undergoes rearrangements to irreversible AGEs. AGEs are a mixture of different moieties. When oxidation is involved in their formation, so-called glycoxidation products such as pentosidine and N’(carboxymethyl)lysine result. Initially, AGEs were thought to form only on long-lived extracellular molecules, because of the slow rate of reaction of glucose with proteins. However, intracellular and short-lived molecules have now also been shown to be targets for AGE formation through reactions with other sugars such as glucose 6-phosphate and glyceraldehyde 3-phosphate, which form AGEs at a much faster rate than glucose. In addition, the highly reactive dicarbonyl compounds methylglyoxal, glyoxal and 3-deoxyglucosone, which are formed from the degradation of glycolytic intermediates, are believed to contribute importantly to the formation of AGEs in vivo [104]. This so-called carbonyl stress has been implicated in the accelerated vascular damage in both diabetes and uraemia. In endothelial cells, methylglyoxal is probably the main AGE formed [105,106].

The introduction of AGEs in the extracellular matrix can interfere with endothelial function in several ways. AGE-modified type I and IV collagen inhibit normal matrix formation and cross-linking, and decrease arterial elasticity, AGE-modified matrix stimulates interactions with mononuclear cells and macromolecules such as LDL, and AGEs may act as oxidants. In addition, AGE-modified plasma proteins can bind to AGE receptors, including RAGE (receptor for AGE), the macrophage scavenger receptor A, galectin-3, AGE-R1/p60 and AGE-R2/p90, on different cell types such as endothelial cells [107]. Ligation of RAGE has been shown to mediate signal transduction via a receptor-mediated induction of ROS and activation of the transcription factors NF-κB and p21^{cst}. In endothelial cells, the expression of the thrombomodulin, tissue factor and VCAM-1 genes is adversely affected; in macrophages and mesangial cells, there is an increased expression of cytokines and growth factors such as IL-1, TNF-α and TGF-β. In animal models, blockade of RAGE inhibited the development of macrovascular disease and diabetic nephropathy.

Many aspects of diabetic complications are thus potentially related to the effect of Amadori-adducts and AGEs [108–113]. Clinical trials with aminoguanidine, an AGE formation inhibitor that had shown promise in animal experiments, have unfortunately been halted because of unforeseen side effects [114], but trials with other AGE formation inhibitors and with AGE cross-link breakers are underway [115].

Hyperglycaemia-induced oxidative stress as a common activator of the four biochemical pathways

Recent evidence suggests that hyperglycaemia-induced mitochondrial overproduction of superoxide anion radicals plays a key role in the activation of the above pathways [77] (Figure 3). The overproduction of superoxide, in particular by mitochondria that have been uncoupled by the flux of NADH from the hyperglycaemia-enhanced glycolysis, causes inhibition of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and subsequent accumulation of glycolysis intermediates. Hyperglycaemia-induced GAPDH inhibition was found to be a consequence of poly(ADP-ribosyl)ation of GAPDH by PARP [poly(ADP-ribose) polymerase], which was activated by DNA strand breaks produced by mitochondrial superoxide overproduction [116]. Since inhibition of hyperglycaemia-induced ROS production prevented the hyperglycaemia-associated activation of the aldose reductase and hexosamine pathways, PKC activation and AGE formation by methylglyoxal, oxidative stress may be the initial event in endothelial cell dysfunction [77]. Further support for the involvement of accumulated glycolysis intermediates came from experiments demonstrating that activation of the enzyme transketolase by benfotiamine reduced these glycolysis intermediates and the various pathways of endothelial activation, and prevented experimental diabetic microvascular retinopathy and nephropathy [78,117].

In addition to mitochondrial uncoupling, several other mechanisms can contribute to superoxide production in diabetes, namely auto-oxidation of glucose and non-enzymatic glycation [118], activation of NADPH oxidases and uncoupling of eNOS (endothelial NOS) [119–121], and impaired antioxidant status [122]. eNOS may become uncoupled in the presence of low levels of 1-arginine or cofactors, thus leading to the generation of superoxide instead of NO.

Oxidative stress as a final common pathway of hyperglycaemia-induced vascular dysfunction

Enhanced oxidative stress in hyperglycaemia is indicated by increased levels of lipid hydroperoxides [123,124] and urinary excretion of 8-iso-PGF_2α (8-iso-prostaglandin F_2α) [125]. ROS can affect many signalling pathways, such as G-proteins, protein kinases, ion channels and transcription factors, and may modify endothelial function by a variety of mechanisms [126]. These include direct effects on the endothelium such as peroxidation of membrane lipids, activation of NF-κB and interference with the availability of NO. It is not known whether oxidative stress causes endothelial dysfunction in human diabetes. Although in short-term experiments, high doses of vitamin C can improve some aspects of endothelial
dysfunction in diabetes [127], randomized clinical trials with antioxidants have failed to show a decrease in cardiovascular disease [128,129]. In diabetic patients, long-term treatment with high doses of vitamin E has no beneficial effects on endothelial or left ventricular function [130]. Because vitamin E-treated patients had a worsening in some vascular reactivity measurements when compared with control subjects, the use of high dosages of vitamin E cannot be recommended.

Growth factors and cytokines
There is solid evidence that TGF-β [with other growth factors such as IGF-1 (insulin-like growth factor-1) and EGF (epidermal growth factor)] plays a major role in diabetic nephropathy [131], and VEGF is important in severe retinopathy [132]. TNF-α may cause insulin resistance and impair endothelial function in large arteries and also in the microcirculation, suggesting that it may be important both in atherothrombosis and in microangiopathy.

TGF-β
TGF-β1 is secreted by different cell types as an inactive dimer consisting of a latency-associated peptide and mature TGF-β1, and is activated by serine proteases such as plasmin. TGF-β1 is increased through hyperglycaemia-induced PKC activation, Amadori-albumin and AGEs, stretch and Ang II, and cytokine activation of endothelial cells [133]. TGF-β1 exerts its effects via two transmembrane serine/threonine kinase receptors, type I and II, that are co-expressed on mesangial and endothelial cells [134]. TGF-β1 regulates multiple cellular functions, including inhibition and stimulation of cell growth, cell death or apoptosis and cellular differentiation. It is also a potent inducer of extracellular matrix protein synthesis such as type I and IV collagen, fibronectin, laminin and proteoglycans. Enhanced glomerular expression of TGF-β1 in human diabetic nephropathy has been reported [135]. TGF-β1 has been proposed as the major candidate to mediate the progression of diabetic nephropathy [136] by inducing glomerular and tubular changes resulting in progressive thickening of the glomerular basement membrane, expansion of the mesangial matrix [135], a reduced glomerular filtration function and an increased excretion of proteins [137]. Furthermore, treatment of diabetic mice with anti-TGF-β1 antibodies significantly attenuated the increase in TGF-β1 activity and extracellular matrix expression [138], demonstrating a role for TGF-β1 in diabetic nephropathy. The role of TGF-β in the thickening of capillary basement membranes elsewhere (e.g. in the retina) is less well established. TGF-β1 also has potent anti-inflammatory effects on vascular cells, down-regulating cytokine-induced expression of VCAM-1 [139] and MCP-1 [140].

VEGF
VEGF is a multi-tasking cytokine, which stimulates differentiation, survival, migration, proliferation, tubulogenesis and vascular permeability in endothelial cells [141]. The expression of VEGF can be induced by hypoxia through HIF-1 (hypoxia-inducible factor-1), but also by IGF-1 and TGF-β1 [142]. VEGF binds to several receptors, of which VEGF receptors 1 and 2, also known as fms-like tyrosine kinase and fetal liver kinase-1, have been best characterized [143]. In diabetic retinopathy, alteration of retinal microvasculature and increased vascopermeability are strongly stimulated by the interaction of VEGF with the endothelium [144]. Several studies have shown increased vitreal VEGF levels in patients with proliferative diabetic retinopathy compared with patients without proliferative retinopathy [145], and antagonists of VEGF and its receptors have been shown to reduce retinopathy in animal models [146]. Furthermore, prevention of Ang II formation was associated with lower VEGF concentrations in the vitreous fluid of patients with proliferative diabetic retinopathy [147]. The role of increased or decreased VEGF in other diabetic complications is the subject of much ongoing research [148–150].

TNF-α
TNF-α is an inflammatory cytokine produced by neutrophils, macrophages and, importantly, adipocytes. TNF-α can induce other powerful cytokines such as IL-6 which, in turn, regulates the expression of CRP (C-reactive protein). These mediators alone or in combination can impair endothelial function and contribute to atherothrombosis. In addition, TNF-α can induce insulin resistance, which may at least in part explain why insulin resistance, endothelial dysfunction and atherothrombosis are so closely related (see above). Finally, recent studies have shown that TNF-α and inflammation in general can contribute to the pathogenesis of diabetic nephropathy.

The spectrum of endothelial cell responses elicited by cytokines is varied. Briefly, inflammatory cytokines increase vascular permeability, alter vasoregulatory responses, increase leukocyte adhesion to endothelium and facilitate thrombus formation by inducing pro-coagulant activity, by inhibiting anti-coagulant pathways and by impairing fibrinolysis via stimulation of PAI-1. Activation of the transcription factor NF-κB is crucial in cytokine regulation of gene expression in endothelial cells [151]. NF-κB consists of a family of transcription factors that serve as important regulators of the inflammatory response and the regulation of vascular cell function. NF-κB is activated not only by TNF-α and IL-1, but also by hyperglycaemia, AGEs, Ang II, oxidized lipids and insulin. Examples of NF-κB-regulated genes include VCAM-1, E-selectin, ICAM-1 (intercellular cell-adhesion molecule-1), IL-1, -6 and -8, tissue factor, PAI-1 and inducible NOS. On the other hand, NF-κB
protects against apoptosis and activates the antioxidant enzyme SOD (superoxide dismutase). Taken together, these data suggest that NF-κB pathway is an important contributor to the pathogenesis of vascular disease in diabetes mellitus.

**CRP**

An important downstream marker of inflammation is CRP. Plasma levels of CRP are increased in both Type I and Type II diabetes [45,152]. Numerous studies have shown that CRP levels predict cardiovascular disease [153]. Interestingly, much in vitro data, largely in endothelial cells, but also in monocytes/macrophages and vascular smooth muscle cells, have now suggested a role for CRP in atherogenesis [154,155]. The pro-inflammatory pro-atherogenic effects of CRP that have been documented in endothelial cells include the following: decreased NO and prostacyclin, and increased ET-1, cell adhesion molecules, MCP-1, IL-8 and PAI-1. In monocytes/macrophages, CRP induces tissue factor secretion, increases ROS and pro-inflammatory cytokine release, promotes monocyte chemotaxis and adhesion, and increases oxidized LDL uptake. Also, CRP has been shown in vascular smooth muscle cells to increase inducible NO production, increase NF-κB and MAPK (mitogen-activated protein kinase) activities, and, most importantly, up-regulate Ang II type 1 receptor, resulting in increased ROS and vascular smooth muscle cell proliferation. Future studies should be directed at delineating the molecular mechanisms for these important in vitro observations. Also, studies should be directed at confirming these findings in animal models and other systems as proof of concept. In conclusion, CRP is a risk marker for cardiovascular disease and could emerge as a mediator in atherogenesis.

**The metabolic syndrome: insulin resistance, insulin, insulin precursor molecules, hypertension, dyslipidaemia and obesity**

**Insulin resistance**

How metabolic and endothelial insulin resistance occur and why they are closely related is not fully understood (see above). Endothelial insulin resistance, whether primary or secondary (Figure 2), can be regarded as a form of endothelial dysfunction and conceivably contributes to both atherothrombosis and microangiopathy. Both TNF-α and NEFAs can cause metabolic and endothelial insulin resistance. TNF-α may induce endothelial insulin resistance through its ability to impair intracellular signaling by inhibition of insulin-stimulated autophosphorylation and phosphorylation of IRS-1 (insulin receptor substrate-1). How NEFAs impair insulin's endothelial actions is not clear.

**Insulin**

Both Type I and Type II diabetes are usually accompanied by chronic hyperinsulinaemia. For the vasculature, both vasodilator and vasoconstrictor effects of insulin have been described [156]. NO is the main mediator of the acute dilatory effects of insulin in human, whereas the vasoconstrictor effects of insulin are mainly mediated by the vasoconstrictor peptide ET-1. Insulin stimulates NO production in endothelial cells by subsequently activating the intracellular enzymes PI3K (phosphoinositide 3-kinase) and Akt [157], which phosphorylates and activates eNOS, whereas ET-1 production is MAPK dependent [73]. An imbalance between the release of NO and ET-1 may be involved in the pathophysiology of hypertension and also atherosclerosis in insulin-resistant states associated with endothelial dysfunction. However, whether insulin has atherogenic effects is controversial, mainly because it is not clear whether effects such as increased vascular permeability to macromolecules and increased vascular smooth muscle cell proliferation occurs at physiological concentrations.

**Insulin precursor molecules**

Type II diabetes is characterized by high levels of molecules that arise as a by-product of insulin synthesis and secretion, notably intact pro-insulin, pro-insulin split products and C-peptide. In contrast, levels of these peptides are abnormally low in Type I diabetes. The vascular effects of these peptides have not been extensively investigated. Insulin precursors may increase plasma levels of PAI-1. C-peptide may affect vascular tone and permeability, although the molecular basis for these effects has not been fully elucidated [158,159].

**Hypertension**

Hypertension is a major determinant of microangiopathy and atherothrombosis in diabetes [160]. Hypertension causes endothelial activation as indicated by elevated levels of soluble adhesion molecules [161] and impaired NO availability (see above); whether the latter then contributes to increased blood pressure is not clear. Experimental data indicate that a decrease NO availability in the kidney may contribute to vasoconstriction and decreased glomerular filtration, impaired tubuloglomerular feedback, decreased medullary blood flow and impaired pressure natriuresis, and progressive proteinuria. Salt sensitivity of blood pressure may denote an inability to increase NO availability in response to increased blood pressure [162]. Impaired endothelial function in secondary hypertension is usually reversible upon reduction of blood pressure; in contrast, some patients with essential hypertension do not normalize endothelial function with blood-pressure lowering [162,163]. Data on this issue in diabetes are scarce.

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Dyslipidaemia

One important cardiovascular risk factor in Type II diabetic people is dyslipidaemia [164]. This is characterized by low HDL-cholesterol and a preponderance of small-dense LDL. Even slight elevations of LDL-cholesterol in Type II diabetic patients are associated with a substantial increase in cardiovascular risk. In addition, Type II diabetes, especially when glycemic control is poor, is characterized by increased postprandial triacylglycerol-rich lipoproteins (chylomicrons and very-LDL particles), which can enhance oxidative stress and impair endothelial function directly and indirectly by increasing the production of small-dense LDL particles and by reducing HDL [165]. These changes contribute to atherothrombosis and may also play a role in nephropathy [166]. Dyslipidaemia has been associated with increases in urinary albumin excretion in both Type I and Type II diabetes [167,168].

Obesity

The current obesity epidemic implies that obesity is becoming an increasingly important risk factor for cardiovascular disease [169]. This is the case not only for large-artery disease, such as myocardial infarction and stroke [170], but also for disease entities that are caused wholly or in part by microangiopathy, notably retinopathy, nephropathy and heart failure [171–173]. How obesity causes large-artery disease and microangiopathy is poorly understood. In part, these may be the consequences of obesity-associated hypertension, insulin resistance and dyslipidaemia. However, these risk factors cannot entirely explain the association of obesity with large-artery disease and microangiopathy. de Jongh et al. [174] have recently demonstrated that impaired microvascular function may contribute to the development of obesity-associated microangiopathy, hypertension and insulin resistance. The pathophysiological mechanism behind the relationship between obesity and microvascular dysfunction is probably multifactorial and may include mediators directly secreted by adipocytes, such as NEFAs [175], TNF-α (see above) and adiponectin, which can influence microvascular function.

Adiponectin is highly expressed in adipose cells. Plasma adiponectin levels are reduced in patients with obesity [176], as well as in patients with some of the disease states frequently associated with obesity such as Type II diabetes mellitus [177,178] and also in patients with coronary artery disease [179]. In contrast with studies in Type II diabetic patients, small cross-sectional studies in Type I diabetic patients found increased plasma levels of adiponectin [180]. In addition to important roles in the regulation of energy homeostasis and insulin sensitivity [181], adiponectin has recently been shown in vitro to modulate a wide array of biological functions with anti-atherogenic properties, including the inhibition of the expression of adhesion molecules [182] and reduction of monocyte attachment to endothelial cells [183], stimulation of the production of NO [184] and suppression of TNF-α in macrophages [185]. In humans, hypoadiponectinaemia is associated with impaired vasoreactivity [186,187]. The inverse relationship of adiponectin with CRP [188,189] also suggests that decreased production of adiponectin contributes to vascular complications.

Recently, evidence has been provided that an increased adiponectin concentration is associated prospectively with a lower risk of coronary artery disease in Type I diabetes and that this effect was independent of conventional risk factors and markers of inflammation and insulin resistance [190]. Taken together, these data strongly indicate that hypoadiponectinaemia is linked to inflammation, endothelial damage and vascular diseases.

CONCLUSIONS

Assessment of blood flow, vascular reactivity and several markers of endothelial dysfunction have been shown to be associated with an adverse cardiovascular prognosis regardless of the presence of diabetes. In diabetes, the close link between endothelial dysfunction and (micro)albuminuria is an attractive explanation for the fact that microalbuminuria is a risk marker for atherothrombosis. Although endothelial dysfunction predicts the occurrence of microalbuminuria, causality needs to be determined.

In diabetes, hyperglycaemia and components of the metabolic syndrome cause endothelial dysfunction directly or indirectly. A common mechanism underlying endothelial dysfunction relates to an increase in oxidative stress. New insights into mechanisms of endothelial dysfunction may lead to novel important strategies of treatment. Since microvascular endothelial dysfunction is closely associated with and may contribute to insulin resistance, hypertension and microalbuminuria, improvement of microvascular function should be one of the first targets.

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