

Endothelial dysfunction in diabetes mellitus

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Abstract: Diabetes mellitus is associated with an increased risk of cardiovascular disease, even in the presence of intensive glycemic control. Substantial clinical and experimental evidence suggest that both diabetes and insulin resistance cause a combination of endothelial dysfunctions, which may diminish the anti-atherogenic role of the vascular endothelium. Both insulin resistance and endothelial dysfunction appear to precede the development of overt hyperglycemia in patients with type 2 diabetes. Therefore, in patients with diabetes or insulin resistance, endothelial dysfunction may be a critical early target for preventing atherosclerosis and cardiovascular disease. Microalbuminuria is now considered to be an atherosclerotic risk factor and predicts future cardiovascular disease risk in diabetic patients, in elderly patients, as well as in the general population. It has been implicated as an independent risk factor for cardiovascular disease and premature cardiovascular mortality for patients with type 1 and type 2 diabetes mellitus, as well as for patients with essential hypertension. A complete biochemical understanding of the mechanisms by which hyperglycemia causes vascular functional and structural changes associated with the diabetic milieu still eludes us. In recent years, the numerous biochemical and metabolic pathways postulated to have a causal role in the pathogenesis of diabetic vascular disease have been distilled into several unifying hypotheses. The role of chronic hyperglycemia in the development of diabetic microvascular complications and in neuropathy has been clearly established. However, the biochemical or cellular links between elevated blood glucose levels, and the vascular lesions remain incompletely understood. A number of trials have demonstrated that statins therapy as well as angiotensin converting enzyme inhibitors is associated with improvements in endothelial function in diabetes. Although antioxidants provide short-term improvement of endothelial function in humans, all studies of the effectiveness of preventive antioxidant therapy have been disappointing. Control of hyperglycemia thus remains the best way to improve endothelial function and to prevent atherosclerosis and other cardiovascular complications of diabetes. In the present review we provide the up to date details on this subject.

Keywords: endothelial dysfunction, diabetes mellitus, hyperglycemia, insulin resistance, microalbuminuria

Introduction

Isolated diabetes mellitus is a frequent and increasing public health problem. Importantly, diabetes has a prevalence of 2%–5% in most Western countries, and is rapidly increasing in Asiatic countries due to changes in dietary habits during the last years (Contreras et al 2000).

Over the last two decades it has become evident that the endothelium is not an inert, single-cell lining covering the internal surface of blood vessels, but in fact plays a crucial role in regulating vascular tone and structure. Importantly, a healthy endothelium inhibits platelet and leukocyte adhesion to the vascular surface and maintains a balance of profibrinolytic and prothrombotic activity (Libby 2002).

Endothelial dysfunction has received increasing attention as a potential contributor to the pathogenesis of vascular disease in diabetes mellitus. Under physiological conditions, there is a balanced release of endothelial-derived relaxing and

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contracting factors, but this delicate balance is altered in diabetes and atherosclerosis, thereby contributing to further progression of vascular and end-organ damage (Tan, Chow, Ai et al 2002).

Hyperglycemia is the major causal factor in the development of endothelial dysfunction in diabetes mellitus. Although the mechanisms underlying this phenomenon are likely to be multifactorial. Insulin resistance has been described in several diseases that increase cardiovascular risk and mortality, such as diabetes, obesity, hypertension, metabolic syndrome, and heart failure.

Increasing evidence suggests that the progression of insulin resistance to type 2 diabetes parallels the progression of endothelial dysfunction to atherosclerosis. Insulin resistance is closely linked with visceral adiposity, and early data suggested that free fatty acids were responsible for this association (Boden and Shulman 2002). More recently, other plasma biomarkers produced by adipose tissue, including TNF and resistin, have been shown to have elevated levels during obesity and to mediate insulin resistance. Conversely, the expression and secretion of adiponectin, an adipocyte-specific protein that enhances insulin-mediated glucose uptake, is inversely correlated with fat mass (Lyon et al 2003). Several studies have demonstrated that nitric oxide (NO)-mediated vasodilation is abnormal in patients with type 2 diabetes (Williams et al 1996). Brachial artery responses were found to be abnormal to both endogenous and exogenous NO donors, suggesting that there was increased inactivation of NO, possibly caused by enhanced metabolism of NO or abnormal vascular smooth muscle cell (VSMC) responses to NO because of alterations in signal transduction in the guanylate cyclase pathway. Obese patients without frank type 2 diabetes have been shown also to have abnormal endothelial function (Steinberg et al 1996; Perticone et al 2001). Herein, we review the literature about endothelial dysfunction in diabetes mellitus with regards to its pathogenesis at molecular and clinical level, and possible available mode of therapy.

Normal endothelial cell (EC) function

The EC is no longer considered a simple barrier. In fact it is a complex organ, with paracrin and autocrin function, which provides a "first line" physiological defense against atherosclerosis. The EC lines the internal lumen of all the vasculature and serves as an interface between circulating blood and vascular smooth muscle cells (VSMC). In addition to serving as a physical barrier between the blood and tissues,

the EC facilitates a complex array of functions in intimate interaction with the VSMC, as well as cells within the blood compartment (Vanhoutte 1995; De Meyer and Herman 1997; Haller 1997; Mombouli 1997).

The last two decades of research have established unambiguously that the EC has a critical role in overall homeostasis whose functions are integrated by a complicated system of chemical mediators. This system exerts effects on both the surrounding VSMC and the cells in the blood that lead to one or more of the following alterations: (1) vasodilatation or vasoconstriction to regulate organ blood, (2) maintenance of fluidity of blood and avoidance of bleeding, (3) proinflammatory or anti-inflammatory changes, and (4) growth and/or changes in the phenotypic characteristics of VSMC (Wautier et al 1983, 1990, 1996; Conger 1994; Chappey 1996; De Meyer and Herman 1997).

During the last decade, a multitude of experimental arguments have led to the concept that NO is not only involved in the control of vasomotor tone but also in vascular homeostasis and neuronal and immunological functions. Endogenous NO is produced through the conversion of the amino acid, L-arginine to L-citrulline by the enzyme, NO-synthase (NOS) from which several isoforms have recently been isolated, purified, and cloned. NOS-type I (isolated from brain) and type III (isolated from ECs) are termed "constitutive-NOS" and produce picomolar levels of NO from which only a small fraction elicits physiological responses. NO produced by NOS type III in the endothelium diffuses to the vascular smooth muscle (VSM) where it activates the enzyme guanylate cyclase. The concomitant increase in cyclic GMP then induces relaxation of the VSM.

The EC produces mediators that induce vasoconstriction, including endothelin (Haefliger et al 1992; Cacoub 1993; Levin 1996), prostaglandins (Viberti 1989; Goldin et al 1996) and angiotensin II (ANG-II) (Studdy et al 1983; Hsueh and Anderson 1993; Rabelink and Bakris 1998; McFarlane 1999) and regulates vascular tone by maintaining a balance between vasodilation (NO production) and vasoconstriction (eg, A-II generation). ANG-II is produced in local tissues by the EC (Toop et al 1989; Mombouli 1997) and exerts regulatory effects upon several VSMC functional activities including contraction (ie, vasoconstriction), growth, proliferation, and differentiation. NOS also are regulated by local concentrations of bradykinin (Busse et al 1993). This peptide acts with b2 receptors on the EC cell surface membrane, increasing the generation of NO via NOS activation. Interestingly, the local concentrations of bradykinin are regulated by the activity of angiotensin converting enzyme (ACE). ACE breaks

down bradykinin into inactive peptides (Luscher et al 1993; Mombouli 1997).

Furthermore the EC has a prominent role in maintaining blood fluidity and restoration of vessel wall integrity to avoid bleeding and plays a key role in the balance between the coagulation and fibrinolytic systems. In addition to its key role in growth and differentiation of the VSMC through the release of either promoters of growth and/or inhibitors of growth and differentiation and, as such, has an impact on vascular remodeling (Cowan and Langille 1996). However, strong evidence suggests that promotion of VSM growth is mediated by local production of platelet growth factor (PGF) and ANG-II (Williams 1998).

The EC is also involved in the production of specific molecules that have a regulatory role in inflammation such as leukocyte adhesion molecule (LAM), intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM). These molecules are denominated “adhesion molecules” and function to attract and “anchor” those cells involved in the inflammatory reaction. Very recently it has been demonstrated that the atherosclerotic process is associated with an increased blood level of inflammation (acute phase proteins) markers (Tracy 1997; Biegelsen and Loscalzo 1999).

Endothelial dysfunction and microalbuminuria

Microalbuminuria is usually defined as a urinary albumin excretion rate of 30–300 mg in a 24 h urine collection, or as a urinary albumin excretion rate of 20–200 mg/min in a timed overnight urine collection, although microalbuminuria was demonstrated to be a predictor for cardiovascular events at levels below these conventional cut-off values. It is an independent risk factor for the development of cardiovascular disease and a predictor of cardiovascular mortality in the diabetic population. It is associated with insulin resistance, atherogenic dyslipidaemia, and central obesity, the absence of nocturnal drop in both systolic and diastolic pressures and is a part of the metabolic cardiovascular syndrome associated with hypertension. Because microalbuminuria is related to endothelial dysfunction and increased oxidative stress, it is not surprising that diabetic atherosclerosis parallels diabetic glomerulosclerosis and is a very powerful risk factor for coronary heart disease and stroke in diabetic persons. The impairment of endothelium and NO-dependent vasodilation in the human forearm microcirculation in type 1 diabetic patients is more severe in the presence of microalbuminuria (Dogra et al 2001). This defect was attributed to the presence of advanced

glycosylation end products and/or increased generation of the superoxide anion in type 1 diabetic patients, both of which are known to effectively antagonize the biological activity of NO. Furthermore, basal NO-dependent vasodilatory tone has been found to be reduced in type 1 diabetic patients. Impaired NO biosynthesis has been demonstrated in the forearm vasculature of type 1 diabetic patients with microalbuminuria (Elliot 1993).

A cross-sectional association possibly exists between increased urinary albumin excretion and the presence of sub-clinical atherosclerosis (Jensen 2000), whether urinary albumin excretion increases before, during or after the development of morphological changes in the atherosclerotic process (in stage I, II or III), ie, whether microalbuminuria is a predictor of atherosclerosis or of atherosclerotic progression is not clear. Multiple markers of endothelial dysfunction have also been documented in normoalbuminuric subjects with type 2 diabetes, suggesting that the vasculopathy in type 2 diabetes occurs early and may even be operative before the development of microalbuminuria (Lim et al 1999).

Endothelial dysfunction and the metabolic syndrome

The metabolic syndrome is a highly prevalent multifaceted clinical entity produced through the interaction of genetic, hormonal and lifestyle factors. A distinctive constellation of abnormalities precedes and predicts the accelerated development of inflammation and coagulation represent emerging risk contributors associated with obesity and insulin resistance, central components of the metabolic syndrome, which act in concert with traditional abnormalities to increase cardiovascular risk. The initiation and progression of atherosclerosis may have its origins in impaired endothelial function that can be detected at the earliest stages of development of the syndrome. The basic elements of the metabolic syndrome and accelerated phase of atherogenesis are often silent partners that present many years before the onset of type 2 diabetes mellitus. The ability to detect and monitor sub clinical vascular disease, as a reflection of the multiple factors that contribute to impair arterial wall integrity, holds potential to further refine cardiovascular risk stratification (McVeigh and Cohn 2003).

Over time, insulin resistance is associated with more components of the metabolic syndrome (Table 1) including, low high density lipoprotein (HDL) cholesterol level, hypertension, increased vascular production of reactive oxygen species, increased plasma PAI-1-mediated thrombotic tendency, hyperuricemia, high triglyceride levels

Table 1 Clinical identification of the metabolic syndrome

| Risk factor | Defining level |
|--|----------------------------------|
| Abdominal obesity*(waist circumference)† | Men >102 cm Women >88 cm |
| Triglycerides | ≥150 mg/dL |
| High-density lipoprotein cholesterol | Men <40 mg/dL Women <50 mg/dL |
| Blood pressure | ≥130/85 mm Hg |
| Fasting glucose | ≥110 mg/dL |

Diagnosis is made when three or more of the risk determinants are present (Hsueh et al 2004).

and elevation of oxidation-prone small dense low-density lipoprotein (LDL) cholesterol levels. All are associated with endothelial dysfunction (McIntyre et al 1999; Toikka et al 1999; Kita et al 2001; Matsuoka 2001). The incidence of the metabolic syndrome, as defined by the Adult Treatment Panel III report, increases with age. Carbohydrate intolerance occurs when the islet cells can no longer make enough insulin to overcome insulin resistance, and thus secreted insulin does not appropriately suppress hepatic glucose production. Free fatty acids may contribute to these changes, given that they can induce islet cell apoptosis and enhance liver gluconeogenesis (Arner 2003).

Free fatty acids and proinflammatory adipokines likely contribute to endothelial dysfunction early in the course of insulin resistance. Free fatty acids induce endothelial dysfunction, whereas several adipokines promote both inflammatory responses and insulin resistance (Steinberg et al 1997). The role of tumor necrosis factor TNF- in vascular inflammation is well known, and a role for leptin is emerging (Fantuzzi and Faggioni 2000; Plutzky 2001). Adiponectin attenuates vascular inflammatory changes, but its effects are reduced by its diminished expression in patients who are obese or have insulin resistance (Ouchi et al 2000, 2001; Yokota et al 2000). In the later stages of the metabolic syndrome, hyperglycemia contributes to the pathology by affecting signal transduction pathways, including those mediated through protein kinase C, in the endothelial cells (Teschfariam et al 1999).

Insulin resistance in the natural history of type 2 diabetes

Insulin resistance, defined as the decreased ability of insulin to promote glucose uptake in skeletal muscle and adipose tissue and to suppress hepatic glucose output, may be present for many years before the development of any abnormality in plasma glucose levels (Kahn and Flier 2000; Haffner 2003).

The insulin resistance syndrome encompasses more than a subnormal response to insulin-mediated glucose disposal.

Patients with this syndrome also frequently display elevated blood pressure, hyperlipidemia and dysfibrinolysis even without any clinically demonstrable alteration in plasma glucose concentrations. Of note, endothelial dysfunction also has been demonstrated in patients with hypertension (Landin et al 1990; Luscher 1990; Bonner 1994; Briner and Luscher 1994; Kamide et al 1996; Lemne and de Faire 1996; Hedner and Sun 1997; Khder et al 1998), which is one of the features of the insulin resistance syndrome. It is tempting to speculate that loss of endothelial-dependent vasodilation and increased vasoconstrictors might be etiological factors of hypertension. Moreover loss of activity and/or quantity of endothelium-bound protein lipase activity may contribute to hyperlipidemia, which is typical of the insulin resistance syndrome. A synergistic interaction and vicious cycle may exist in which endothelial dysfunction contributes to insulin resistance and vice versa (Figure 1).

Insulin resistance has a well-known but not completely defined genetic influence, frequently transmitted along generations in any given family. Endothelial dysfunction has been demonstrated in insulin-resistant states in animals and humans and may represent an important early event in the development of atherosclerosis. Insulin resistance may be linked to endothelial dysfunction by a number of mechanisms, including disturbances of sub cellular signaling pathways common to both insulin action and NO production. Other potential unifying links include the roles of oxidant stress, endothelin, the renin angiotensin system and the secretion of hormones and cytokines by adipose tissue. Individuals who advance toward the development of type 2 diabetes experience progressive deterioration of glucose tolerance over time. In addition, obesity, which also has an important genetic component, invariably exacerbates any degree of insulin resistance (Kahn and Flier 2000). Thus, obesity and insulin resistance are usually present for many years before the appearance of other abnormalities such as hypertension, dyslipidemia, type 2 diabetes and cardiovascular disease. In certain individuals, obesity and insulin resistance may be present during childhood and adolescence (Goran et al 2003).

Increasing evidence suggest that hyperinsulinaemia is linked with the development of atherosclerosis in patients with diabetes. Whether hyperinsulinaemia directly affects neutrophil transendothelial migration and surface expression of related endothelial adhesion molecules were studied on healthy volunteers and from patients with non-insulin-dependent diabetes mellitus across human umbilical vein. Endothelial cells cultured in insulin-rich medium using

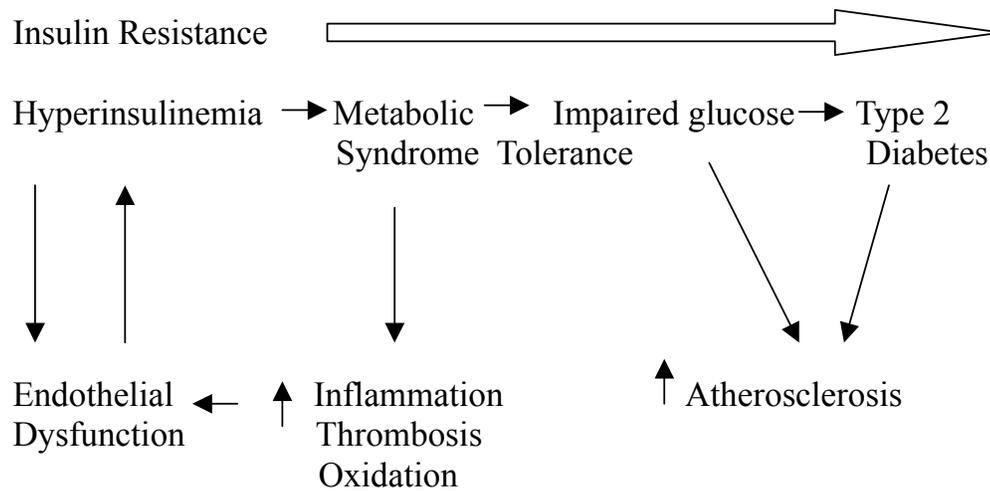


Figure 1 Progression of endothelial dysfunction in relation the progression of insulin resistance (Hsueh et al 2004).

cell-culture inserts high insulin (over 50 microU/ml for 24 h) enhanced neutrophil transendothelial migration in a dose-dependent manner. This was associated with increased expression of platelet endothelial cell adhesion molecule-1 (PECAM-1) but not of intercellular adhesion molecule-1 (ICAM-1), P-selectin or E-selectin (Okouchi et al 2002).

Mechanisms of endothelial dysfunction in insulin resistance

Multiple, interrelated mechanisms contribute to endothelial cell dysfunction in insulin resistance. The exact mechanism by which dyslipidemia contributes to endothelial dysfunction is unknown. It is known that endothelial NOS infiltrates into caveolae, which are cholesterol-rich invaginations present in endothelial cells and VSMC that decrease vasoconstrictive responses to angiotensin II, endothelin and constitutive endothelial NOS activity in animals (Rizzo 1998). Addition of oxidized LDL to cultured endothelial cells disrupts the caveolae complex and is thought to be associated with decreased endothelial NOS activity and endothelial dysfunction (Blair et al 1999; Drab et al 2001). HDL cholesterol can prevent the oxidized LDL-mediated decrease in cholesterol in caveolae, prevent the translocation of endothelial NOS and caveolae from caveolae, and prevent the decrease in responsiveness to acetylcholine (Uittenbogaard et al 2000). These effects occur because HDL cholesterol donates cholesterol to the caveolae complex. These cellular events are consistent with the proatherogenic effects of LDL cholesterol and oxidized LDL cholesterol and the protective effects of HDL cholesterol.

The presence of hypertension and other atherosclerotic risk factors is associated with increased vascular

angiotensin II generation and activity (Dzau 2001). Because angiotensin II and insulin activate a common signaling pathway, increased sensitivity to angiotensin II may occur in the hyperinsulinemic, insulin-resistant state (Gaboury et al 1994). In addition, angiotensin II stimulates intracellular adhesion molecule-1 and monocyte chemo-attractant protein-1 through the MAPK pathway in endothelial cells and VSMC (Chen et al 1998; Tummala et al 1999; Xi et al 1999). It is likely that many of the components of the metabolic syndrome directly alter endothelial vasoreactivity. However, these factors may decrease NO activity through oxidation pathways; the role of oxygen free radical is discussed below in Oxidative stress and endothelial cell dysfunction.

Role of hyperglycemia in diabetic endothelial dysfunction

Hyperglycemia is the major causal factor in the development of endothelial dysfunction in patients with diabetes mellitus (Figure 2). Clinical trials have identified hyperglycemia as the key determinant in the development of chronic diabetic complications. The formation of advanced glycation end products (AGEs) is an important biochemical abnormality accompanying diabetes mellitus and, likely inflammation in general. Although the mechanisms underlying this phenomenon are likely to be multi-factorial, recent in-vivo and in-vitro studies have indicated a crucial role of the diacylglycerol (DAG)-protein kinase C (PKC) pathway in mediating this phenomenon. PKC may have multiple adverse effects on vascular function, including the activation of superoxide-producing enzymes such as the nicotinamide adenine dinucleotide phosphate (NADPH)

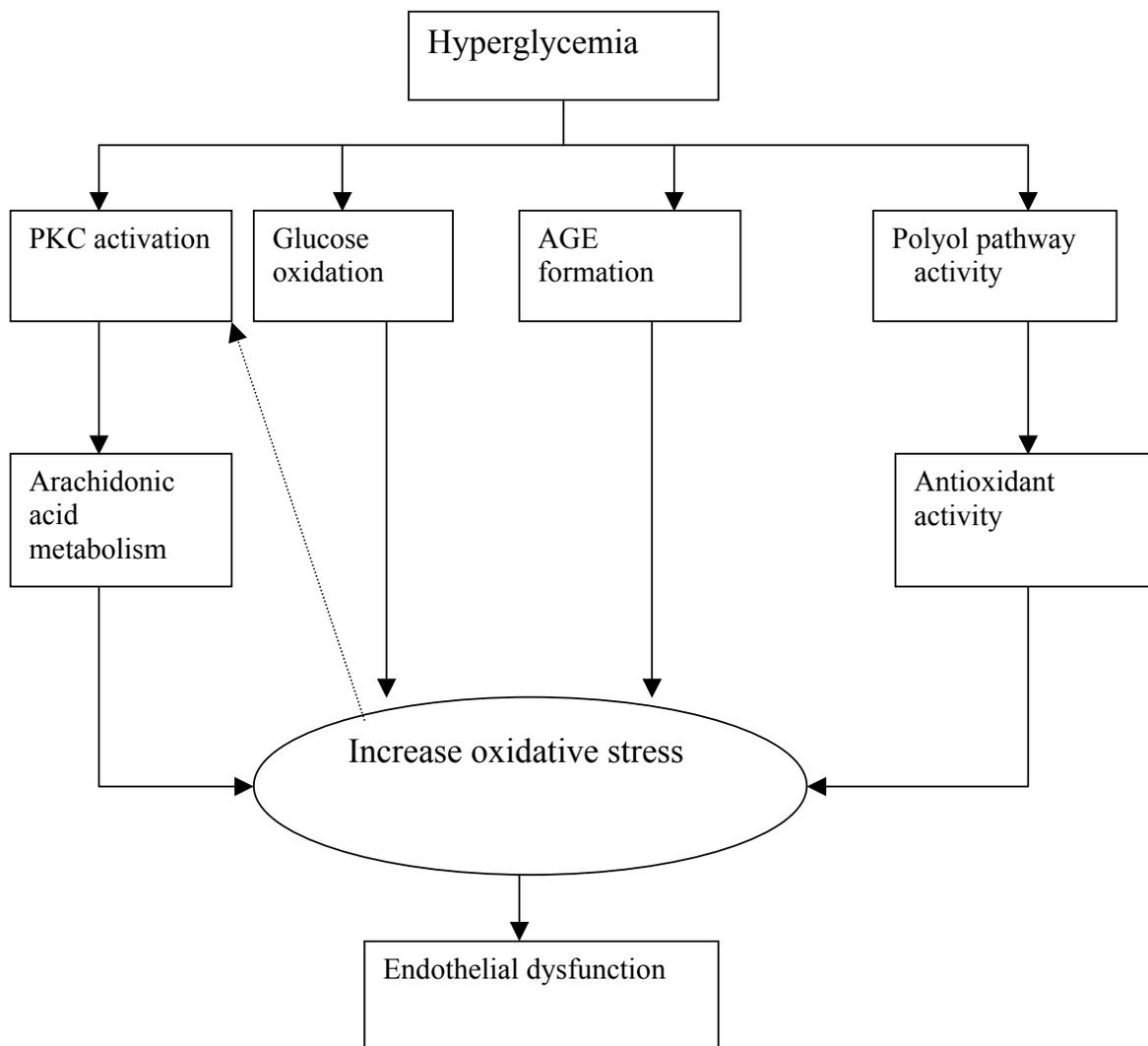


Figure 2 Pathophysiology of hyperglycemia induced endothelial dysfunction (DeVriese et al 2000).

oxidase as well as increased expression of a dysfunctional, superoxide-producing, uncoupled endothelial nitric oxide synthase (NOS III). PKC-mediated superoxide production may inactivate NO derived from endothelial NOS III, and may inhibit the activity and/or expression of the NO downstream target, the soluble guanylyl cyclase. The effects of AGEs on vessel wall homeostasis may account for the rapidly progressive atherosclerosis associated with diabetes. Driven by hyperglycemia and oxidant stress, AGEs form to a greatly accelerated degree in diabetes. Within the vessel wall, collagen-linked AGEs may “trap” plasma proteins, quench NO activity and interact with specific receptors to modulate a large number of cellular properties. On plasma low density lipoproteins (LDL), AGEs initiate oxidative reactions that promote the formation of oxidized LDL. The interaction of AGEs with endothelial, as well as with other

cells accumulating within the atherosclerotic plaque, such as mononuclear phagocytes and smooth muscle cells, provides a mechanism to augment vascular dysfunction. Specifically, the interaction of AGEs with vessel wall component increases vascular permeability, the expression of procoagulant activity and the generation of reactive oxygen species, resulting in increased endothelial expression of endothelial leukocyte adhesion molecules (Wen et al 2002; Hink et al 2003; Basta et al 2004; Farhangkhoei et al 2006), while acute hyperglycemia and hyperinsulinemia induced vasodilatation is not accompanied by changes in microvascular permeability or endothelial markers (Oomen et al 2002).

The effects of acute glycemia on plasma nitric oxide (NO; nitrite plus nitrate) levels, Cu-Zn Superoxide dismutase (Cu-Zn SOD) activity and thiobarbituric acid-reactive substances (TBARS) levels were studied in age-matched female subjects

before and two hours after glucose loading. Plasma NO levels were significantly higher in subjects with diabetic glucose tolerance (DGT) than in subjects with normal glucose tolerance ($p < 0.001$) and impaired glucose tolerance (IGT) ($p < 0.05$) at baseline. TBARS levels were significantly elevated in subjects with DGT and IGT ($p < 0.001$ and $p < 0.001$). Cu-Zn SOD activities were significantly increased in subjects with NGT, and were significantly decreased in subjects with IGT and DGT ($p < 0.001$ and $p < 0.001$) after glucose loading; suggest that NO availability was decreased when the blood glucose levels were only moderately elevated above normal levels. This might be related with the enhanced oxidative stress (Konukoglu et al 2003).

Other studies examined the effect of acute hyperglycemia on endothelium-dependent vasodilation in patients with DM or impaired glucose metabolism in- vivo by plethysmography. The vasodilatory response to acetylcholine at infusion rates of 7.5, 15, and 30 microg/min was studied in the fasting state and at two levels of hyperglycemia, which were achieved by the infusion of glucose, insulin and somatostatin. The vasodilatory response to acetylcholine was measured by calculating the forearm blood flow ratio (FBFR), defined as the measured forearm blood flow at a specific acetylcholine infusion rate divided by the baseline forearm blood flow without acetylcholine infusion. The induction of hyperglycemia resulted in a significant reduction in FBFR for all rates of acetylcholine infusion and suggests the importance of hyperglycemia in the development of endothelial dysfunction observed in patients with DM or impaired glucose metabolism (Bhargava et al 2003; Kim et al 2003).

Oxidative stress and endothelial cell dysfunction

Diabetic endothelium produces an increase in both O₂ and H₂O₂ leading to enhanced intracellular production of OH. Thus, OH is implicated in diabetes-induced endothelial dysfunction (Tesfamariam et al 1992; Pieper et al 1997). Reactive oxygen species (ROS) are generated at sites of inflammation and injury. ROS at low concentrations can function as signaling molecules participating as signaling intermediates in the regulation of fundamental cell activities such as cell growth and cell adaptation responses, whereas at higher concentrations, ROS can cause cellular injury and death. The vascular endothelium, which regulates the passage of macromolecules and circulating cells from blood to tissues, is a major target of oxidative stress, playing a critical role in the pathophysiology of several vascular diseases and disorders. Specifically, oxidative stress increases vascular

endothelial permeability and promotes leukocyte adhesion, which is coupled with alterations in endothelial signal transduction and redox-regulated transcription factors (Hazel et al 2001). Decreased endothelium-dependent vasodilation in diabetic subjects is associated with the impaired action of NO secondary to its inactivation resulting from increased oxidative stress, rather than decreased NO production from vascular endothelium, and that abnormal NO metabolism is related to advanced diabetic microvascular complications (Maejima et al 2001).

Since ROS generation is increased in various disease states including DM and a direct reaction between NO and superoxide anion has been demonstrated, so a hypothesis suggest that inhibition of ROS will restore coronary microvascular responses to ACh in a dog model of DM and topical application of superoxide dismutase (SOD) (250 U/ml) and catalase (250 U/ml) restored to normal ACh induced coronary microvascular responses in DM while having no affect in normal animals (Ammar et al 2000).

How is endothelial function assessed?

Endothelium-dependent vasodilatation can be assessed in the coronary and peripheral circulations. The most relevant methodological issues in the research on endothelial function and dysfunction have recently been published (Deanfield et al 2005; Hadi et al 2005). We provide a summary of the available modalities of testing:

In coronary circulation, non-invasive tests for assessment of coronary endothelial function include Doppler echocardiography, positron emission tomography and phase-contrast magnetic resonance imaging were described. However, the gold-standard test for the evaluation of coronary endothelial function requires invasive coronary angiography; quantitative coronary angiography can be used to examine the changes in diameter in response to intracoronary infusions of endothelium-dependent vasodilators such as acetylcholine. Endothelial function of the coronary microvasculature can be assessed with intracoronary doppler techniques to measure coronary blood flow in response to pharmacological or physiological stimuli (Anderson 1999; Al Suwaidi 2001; Farouque and Meredith 2001). Diagnostic coronary angiography is first performed with a standard femoral percutaneous approach. No nitroglycerin given before the diagnostic approach. Vasomotor responses to acetylcholine and adenosine then assessed (Al Suwaidi et al 2001). After control coronary angiograms had been obtained, a 0.014- Inc Doppler guide wire is introduced through an 8F-guiding catheter into the left anterior descending coronary

artery (LAD). Once baseline flow velocity data are obtained at the position (when a stable doppler signal was obtained), a bolus of intracoronary adenosine (24–36 µg, from a solution of 6 mg adenosine in 1 L of saline) is administered. Then selective intra coronary infusion of increasing concentration of acetylcholine (10^{-6} , 10^{-5} , and 10^{-4} mol/L) is performed for a total 3 minutes through a 2.2 F Ultrafuse coronary infusion catheter. Symptoms, hemodynamic data, electrocardiographic and doppler velocities are recorded at the end of each infusion or bolus injection, followed by selective coronary angiogram. Coronary blood flow is calculated using the formula $D^2 \times APV$, where D represents the coronary diameter and APV equals the average peak velocity from Doppler tracing.

In Peripheral Circulation, Brachial artery ultrasound is a widely used non-invasive measure of endothelial function. Upper-arm occlusion for 5 minutes results in reactive hyperemia after the cuff is released; this increase in shear stress results in endothelium-dependent flow-mediated vasodilatation. Importantly, endothelial dysfunction assessed by this technique correlates with measures of coronary endothelial dysfunction (Anderson et al 1995). Peripheral vascular endothelial function can be assessed by strain-gauge venous impedance plethysmography. This technique examines the change in forearm blood flow in response to direct intra-arterial (brachial artery) administration of agonists. Non-invasive measures of arterial compliance and waveform morphology provide a marker of vascular health (Deanfield et al 2005).

Molecular and cellular basis of endothelial dysfunction in diabetes

Hyperglycemia may lead to intracellular changes in the redox state resulting in depletion of the cellular NADPH pool. Accumulation of AGEs, whose formation is closely linked to oxidative stress, and resultant endothelial dysfunction may start early in the course of type 1 diabetes (Tsukahara et al 2003). Diminished capacity of nitric NOS to generate NO has been demonstrated experimentally when endothelial cells (ECs) were exposed either in-vitro or in-vivo to a diabetic environment (Arbogast et al 1982; Aanderud et al 1985; Koh et al 1985; Lorenziet al 1986; Hattori et al 1991; Nordt et al 1993; Avogaro et al 1999; Cipolla 1999; Salvolini et al 1999).

Over expression of growth factors has also been implicated as a link between diabetes and proliferation of both endothelial cells and vascular smooth muscle, possibly promoting neovascularization. Chronic hyperglycemia leads to non-enzymatic glycation of proteins and macromolecules (Jorge et al 2001). Insulin appears to regulate shedding or

clearance of vascular adhesion protein-1 VAP-1, and an increase in sVAP-1. Therefore; absolute or relative insulin deficiency may be directly involved in the pathogenesis of diabetic angiopathy (Salmi et al 2002).

The diabetic state is typified by an increased tendency for oxidative stress and high levels of oxidized lipoproteins, especially the so-called small dense low density lipoprotein (LDL-C). High levels of fatty acids and hyperglycemia have both been shown to induce an increased level of oxidation of phospholipids as well as proteins. The diabetic state in humans is associated with a prothrombotic tendency as well as increased platelet aggregation; furthermore TNF has been implicated as a link between insulin resistance, diabetes and endothelial dysfunction. The hypothesis has been advanced in recent years that insulin and/or insulin precursors may be atherogenic (Jorge et al 2001). Recent study suggests that E-selectin may enhance CAD prediction beyond traditional risk factors or markers of oxidative stress in Type 1 diabetes (Costacou et al 2005). Furthermore elevated circulating asymmetric dimethylarginine ADMA may contribute to the excess cardiovascular morbidity and mortality in early diabetic nephropathy (Tarnow et al 2004). In addition adverse metabolic stress factors in type 1 diabetes are associated with reduced angiogenicity, endothelial progenitor cells EPC numbers and function (by 44%) compared with age and sex matched control subjects ($P < 0.001$). This reduction was inversely related to levels of HbA (1c) ($R = -0.68$, $P = 0.01$) using an in-vitro angiogenesis assay (Loomans et al 2004).

Role of adhesion molecules has been clarified in the last few years. Elevated level of sICAM-1 in young diabetic patients correlates with metabolic compensation and positive family history of cardiovascular diseases. sVCAM-1 level in diabetic children correlates significantly positively with body mass index (BMI). Evaluation of adhesion molecules levels can be useful tool in the observation of the dynamic development of early phases of atherosclerotic process in young patients with type 1 diabetes (Glowinska et al 2003).

We have reviewed all experimental and clinical studies from 1982–2006 that evaluated endothelial dysfunction in diabetic patients; most of which were prospective studies. Most of these studies in human indicate that endothelial dysfunction is closely associated to microangiopathy and atherosclerosis in diabetic patients.

Endothelial dysfunction in IDDM

The association between diabetes and endothelial dysfunction is particularly true in patients with type 1 diabetes who have either early (microalbuminuria) or late (macroalbuminuria)

nephropathy. A variety of markers indicate endothelial dysfunction including: poor EC-dependent vasodilation, increased blood levels of von Willebrand factor (vWF), thrombomodulin, selectin, PAI-1, type IV collagen and t-PA were demonstrated in this patients population (Yaqoob et al 1993; Dosquet et al 1994; Myrup et al 1994; Makimattila et al 1996; Huszka et al 1997; Cosentino and Luscher 1998; Elhadd et al 1998; Malamitsi et al 1998; Huvers et al 1999). Endothelial dysfunction was an early manifestation of vascular disease in type 2 diabetes but late in the course of type 1 diabetes (Clarkson et al 1996), furthermore studies have shown that the levels of cVCAM-1 were more markedly elevated in type 1 diabetes patients with diabetic retinopathy than in those patients with micro- or macroalbuminuria, whereas no difference in cICAM-1 and cELAM-1 levels was apparent regarding the clinical status of diabetic microangiopathy (Fasching et al 1996). In diabetic subjects, endothelium-dependent vasodilation correlated inversely with serum insulin concentration but not with glucose concentration, glycosylated hemoglobin, or duration of diabetes (Johnston et al 1993). In a 10 year prospective study of 209 insulin-dependent diabetic patients with normal urinary albumin excretion has demonstrated coincidence of microalbuminuria and decreasing high density lipoprotein cholesterol, but no coincidence between onset of microalbuminuria and endothelial dysfunction assessed by von Willebrand factor (Myrup et al 1994; Hadi et al 2005). Little is known about the formation and accumulation of AGEs in young patients with type 1 diabetes. Patients with microalbuminuria ($>$ or $=$ 15 mg/g Cr) showed significantly higher levels of pentosidine and pyrraline, and markers of oxidative stress, 8-hydroxy-2'-deoxyguanosine (8-OHdG) and acrolein-lysine than did normoalbuminuric patients and control. Of these four markers, urinary concentrations of pentosidine, 8-OHdG, and acrolein-lysine were significantly higher in the patients with diabetes than in the healthy control subjects (Tsukahara et al 2003).

In another study a significantly raised mean concentration of a free N-terminal fibronectin 30-kDa domain was found in plasma of diabetic patients with proliferative retinopathy as compared with healthy persons, and a positive correlation was observed between free N-terminal fibronectin and vWF and the degree of albuminuria. No relationship was found between fibronectin and the degree of control of diabetes (Skrha et al 1990). In Participants from the Epidemiology of Diabetes Complications (EDC) cohort, a 10-year prospective study of childhood-onset Type 1 diabetes. Mean age at baseline was 28 years, and diabetes duration was 19 years.

It has been found cellular adhesion molecules E-selectin may enhance CAD prediction beyond traditional risk factors or markers of oxidative stress in Type 1 diabetes (Costacou et al 2005).

Impaired FMD response is a common manifestation in children with type 1 diabetes and is associated with increased carotid artery intima media thickness (IMT). Which may predispose them to the development of early atherosclerosis (Jarvisalo et al 2005). Although small dense LDL and oxidized LDL are features of type 2 diabetes and predict the development of coronary artery disease, their role in type 1 diabetes is less clear. One study has suggested, but do not prove, that LDL particle size and LDL vitamin E may be determinants of conduit and resistance vessel endothelial vasodilator function in type 1 diabetes. Further work will be required to prove cause and effect (Skyrme-Jones et al 2001).

It is well known that pregnant women with diabetes mellitus have a higher incidence of adverse pregnancy outcomes. This issue has been assessed in a study which has found that the arteries obtained from the diabetic pregnant women did not demonstrate any difference in either endothelial or smooth muscle function when compared with non-diabetic pregnant women. The contribution of NO to endothelium-dependent relaxation was approximately 20% in the pregnant women regardless of whether they were diabetic, and approximately 11% in the non-pregnant women (Ang et al 2002). In contrary in another small study the result of which need further confirmation has found that although pregnancy enhances microvascular function, but in women with diabetes, such improvements are insufficient to attain responses seen in healthy non pregnant women. And a persistent vascular defect in young women with type 1 diabetes that may contribute to adverse pregnancy outcome (Ramsay et al 2003).

Endothelial dysfunction, as estimated by plasma vWF concentration, but not fibrinogen, precedes and may predict the development of microalbuminuria in IDDM (Stehouwer et al 1995; Verrotti et al 2003). The presence of endothelial dysfunction in normoalbuminuric diabetic patients suggests it could precede microalbuminuria as an early risk marker for cardiovascular disease (Dogra et al 2001). Recently a small study shows the vasomotor endothelial function in patients with diabetes mellitus (DM) type 1 at different stages of diabetic nephropathy (DN). ie, without renal affection, stage of microalbuminuria (MAU), proteinuria (PU), and chronic renal failure (CRF).in 26 patients with DM type 1 (11 males and 15 females, mean age 25.9 \pm 4.3 years, mean history of

DM 12.9 \pm 3.4 years) and 7 healthy volunteers as control group. Endothelium-dependent vasodilation (EDVD) was assessed using test with reactive hyperemia provoked by 4–5 min occlusion of the brachial artery by pneumocollar and subsequent assessment of arterial diameter changes after decompression using high-resolution ultrasound dopplerography conclude that at the earliest stage of diabetic nephropathy (stage MAU), EDVD is not affected as maximal vasodilation of the brachial artery and endothelial sensitivity to shift tension do not differ from the control values. This means that the stage of MAU is reversible in early treatment, but proteinuria and chronic renal failure are not reversible stages associated with depletion of endothelial cells and loss of sensitivity to changing hemodynamic conditions (Shestakova et al 2003). The role of endothelial dysfunction in the development and progression of cardio-renal syndrome in 93 patients with type 1 diabetes mellitus were studied by same investigator and analyzed endothelial flow-mediated dilation of the brachial artery, levels of endothelin-1, von Willebrand factor, C-reactive protein, renal:albumin and protein excretion rates, glomerular filtration rate (GFR), and cardiovascular functions (ECG, echocardiography, blood pressure monitoring). GFR correlated positively with the coefficient of sensitivity of endothelium to shear stress. Found a positive correlation between BP, permeability of glomerular filter and endothelial dysfunction markers and negative correlation with the coefficient of sensitivity of endothelium to shear stress and GFR. Left ventricle mass correlated with markers of endothelial dysfunction and stage of renal disease (Shestakova et al 2005b).

More severe diabetic nephropathy was associated with higher prevalence of cardiac pathology. Frequency of ischemic heart disease was 13%, 33% and 53%, frequency of left ventricular concentric hypertrophy and remodeling 33%, 40% and 60% among patients with microalbuminuria, proteinuria and chronic renal failure, respectively. Abnormalities of 24-hour blood pressure rhythm as well as signs of endothelial dysfunction were more pronounced in patients with more severe nephropathy. Correlation analysis revealed significant relationships between markers of endothelial dysfunction, parameters of renal function, blood pressure level and mass of left ventricular myocardium. (Shestakova et al 2005a). Low plasma levels of active TGF-beta are associated with an impaired endothelial response and this may provide a useful tool for identifying Type 1 diabetic patients at a greater risk of coronary artery disease (Meeking et al 1999).

It is the general consensus that the occurrence of endothelial cell dysfunction in type 1 diabetes signifies a

very high risk of micro- and macroangiopathy and although the diabetic state predisposes to endothelial cell dysfunction in this disease, is not sufficient to cause it. More likely, other agents (genes, environment) are likely to play a role in determining those patients that will develop aggressive angiopathy and hence endothelial cell dysfunction. Irrespective of whether endothelial cell dysfunction is a cause or a consequence of vascular injury in type 1 diabetes, therapeutic efforts aimed at restoring endothelial cell to normal will more likely have an affect on the natural history of vasculopathy in type 1 diabetes (Jorge et al 2001).

Plasma homocysteine levels in type 1 diabetes and endothelial dysfunction

Although the findings are inconsistent, moderate hyperhomocysteinaemia (15–30 μ mol/l) has been observed in some studies of patients with type 1 diabetes. Adolescent patients with no microvascular complications have lower (Chiarelli et al 2000; Cotellessa 2001; Wiltshire et al 2001) or similar (Pavia et al 2000) homocysteine levels compared with non-diabetic controls. Studies in adult patients have demonstrated similar (Hultberg et al 1991; Chico et al 1998; Vaccaro et al 2000), lower (Robillon et al 1994; Cronin et al 1998) and higher (Hofmann 1998; Targher et al 2000) plasma homocysteine levels compared with non-diabetic controls. The homocysteine levels are independent of vitamin status and reflect the heterogeneous nature of the patients studied, including patients with poor glycaemic control, variable duration of diabetes and a variety of microvascular and macrovascular complications. It would appear that certain subgroups are more likely to be associated with hyperhomocysteinaemia; several factors have suggested: General factors; include genetic, nutrition, increasing age, male gender and renal failure. Factors specific to type 1 diabetes such as a lower age of onset of diabetes, poor glycaemic control, renal hyperfiltration and diabetic nephropathy. The interpretation of the result of these studies should be taken cautiously and further extensive work is needed to finalize this issue.

Endothelial dysfunction in NIDDM

The role of endothelial dysfunction in type 2 diabetes is more complicated than that for type 1. The effects of ageing, hyperlipidemia, hypertension and other factors add to the complexity of the problem. In contrast to patients with type 1 diabetes, endothelial dysfunction can also occur in patients with type 2 diabetes even when the patients have normal urinary albumin excretion. In fact, markers of endothelial

dysfunction are often elevated years before any evidence of microangiopathy becomes evident (Janka 1985; Hsueh and Anderson 1992; Bloomgarden 1998; De Mattia et al 1998; Neri et al 1998; Watts and Playford 1998; Gazis et al 1999). The insulin resistance syndrome encompasses a subnormal response to insulin-mediated glucose disposal and frequently elevated blood pressure, hyperlipidemia and dysfibrinolysis, even without any clinically demonstrable alteration in plasma glucose concentrations (Steinberg et al 1994). There is a growing body of evidence to suggest the coexistence of insulin resistance and endothelial dysfunction. Insulin-induced vasodilation, which is partially mediated by NO release, is impaired in obese individuals who do not have type 2 diabetes but whom display insulin resistance (Steinberg et al 1994; Ferri et al 1997; Cleland et al 2000). Moreover, the obese state, a model of human insulin resistance, is associated with high levels of endothelin in plasma. Also blood concentrations of PAI-1 are high in patients with otherwise uncomplicated obesity (Calles et al 1996). Endothelial activation and acute-phase reaction correlate with insulin resistance and obesity in type 2 diabetic patients (Leinonen et al 2003).

Abnormalities in vascular reactivity and biochemical markers of endothelial cell activation are present early in individuals at risk of developing type 2 diabetes. The vasodilatory responses to acetylcholine were reduced in healthy normoglycemic subjects who have first degree diabetic relatives. The plasma levels of endothelin-1 were significantly higher in subjects with impaired glucose tolerance and patients with type 2 diabetes without vascular complications compared with healthy normoglycemic subjects with no history of type 2 diabetes in a first-degree relative (Caballero et al 1999). In addition there is a significant association between endothelial dysfunction and insulin resistance in young first degree relatives of DM subjects independent of the classic cardiovascular risk factors (Balletshofer et al 2000).

In a case-cohort study, using the Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Research, men and women with elevated levels of sE-selectin had a significantly increased risk of type 2 diabetes after multivariable adjustment. Hazard ratios (95% CIs) comparing tertile extremes of sE-selectin were 2.63 (1.79–3.88) and 1.71 (1.07–2.75) for men and women, respectively. Elevated levels of sICAM-1 were also associated with an increased risk of type 2 diabetes; however, the association was not independent of other diabetes risk factors including E-selectin, while vWF was not associated with risk of type 2 diabetes (Thorand et al 2006). A prospective,

nested case-control study within the Nurses' Health Study, has found elevated E-selectin and ICAM-1 levels can predict incident diabetes in logistic regression models conditioned on matching criteria and adjusted for body mass index (BMI), family history of diabetes, smoking, diet score, alcohol intake, activity index and postmenopausal hormone use. Adjustment for waist circumference instead of BMI or further adjustment for baseline levels of C-reactive protein, fasting insulin, and HbA1c or exclusion of cases diagnosed during the first 4 years of follow-up did not alter these associations (Meigs et al 2004).

In diabetes, glycation, tissue oxidation and endothelial function are all abnormal and predisposing to microvascular complications but interrelationships are complex with glycation appearing most direct (Wen et al 2002). The patients with microalbuminuria, unlike those without it, are characterized by longer course of diabetes, more pronounced lipid exchange disorder, more variable arterial pressure, higher pressure load index, elevated activity of lipid peroxidation (LP) processes and prominent disorder of NO-producing endothelial function. All improve with treatment (Ametov et al 2005). The Endothelium-dependent vasodilation was impaired in the microalbuminuric patients compared with the normoalbuminuria patients and the healthy controls. Plasma PAI-1 and vWF levels increased in the microalbuminuric patients compared with the levels in the normoalbuminuric patients and in the healthy controls (Yu et al 2004).

In type 2 diabetes mellitus as in type 1, increased calpain (calcium-dependent protease) activity in response to hyperglycemia may play a role in diabetic cardiovascular disease. Immunoprecipitation studies revealed that glucose induces loss of NO via a calpain-dependent decrease in the association of hsp90 with endothelial NOS. In addition, inhibition of calpain activity decreased endothelial cell surface expression of the pro-inflammatory adhesion molecules ICAM-1 and VCAM-1 during hyperglycemia (Stalker et al 2003). Furthermore inhibition of PKC activity reduces leukocyte-endothelium interactions by suppressing surface expression of endothelial cell adhesion molecules in response to increased oxidative stress (Booth et al 2002).

In diabetes associated with diabetic microangiopathy, compared with non diabetics, asymmetric dimethylarginine [ADMA]; an endogenous inhibitor of NOS, serum TNF-alpha and soluble TNF receptor I (sTNFR-I) has been assessed in a study which concludes that the serum sTNFR-I and VEGF levels were significantly increased, but no difference in the serum TNF-alpha, sTNFR-II, and ADMA levels

between uncomplicated diabetic patients and in non diabetics (Makino et al 2005).

Increased levels of vWF antigen, t-PA antigen and PAI-1 activity were seen in impaired glucose tolerance (IGT) and diabetics compared with the normal glucose tolerance (NGT). Tissue factor pathway inhibitor TFPI activity and thrombomodulin levels were increased in all elderly subjects, with positive association between HbA (1c), TFPI activity and vWF antigen. Fasting blood glucose levels correlated with vWF antigen, t-PA antigen and PAI-1 activity, whereas urine albumin excretion correlated with TFPI activity, vWF antigen and PAI-1 activity. Serum insulin levels correlated strongly not only with vWF antigen and t-PA antigen but also with PAI-1 activity. This correlation did not change after further adjustment for serum glucose and HbA(1c), which may suggest that in the elderly subjects, impaired fibrinolysis is probably associated with insulin resistance (Leurs et al 2002). The above reflect a prothrombotic state associated with an insulin resistance state, an increased vWF release, raised sP-Sel and TNFalpha levels and, may be, low NO bioavailability, which could lead to a higher risk of development of thrombotic events in hypertensive diabetic patients (Ouvina et al 2001).

NADPH oxidase gene expression is increased in circulating lymphomonocytes from patients with DM, and this increased gene expression is dependent upon metabolic control. Hyperglycemia can mediate its adverse effects through the activation of protein kinase C. Recent study has shown an increase in membrane-associated PKC beta 2 activity in monocytes from patients with DM. This activity was reduced by 40% in the euglycemic condition (Avogaro et al 2006). Further more patients with Type 2 diabetes with good residual C-peptide secretion are better protected from endothelial dysfunction that those with poor C-peptide secretion (Manzella et al 2003).

Endothelial dysfunction may have deleterious effect cardiovascular pathology in diabetic patients. Recent study found a significant association between an abnormal SPECT result, left ventricular diastolic dysfunction and impaired post-ischaeic dilatation of the brachial artery. No association was found between the SPECT result, systolic function and left ventricular hypertrophy, however, an abnormal SPECT result was significantly associated with left ventricular diastolic dysfunction and the deterioration of post-ischaeic dilatation of the brachial artery in asymptomatic patients with type 2 diabetes (Charvat et al 2005). Myocardial blood flow (MBF) measured with positron emission tomography and ^{13}N -ammonia to characterize coronary

circulatory function in states of insulin resistance without carbohydrate intolerance (IR), impaired glucose tolerance (IGT), normotensive and hypertensive type 2 diabetes mellitus (DM) compared with insulin-sensitive (IS) individuals shows that endothelium-dependent coronary vasomotion was significantly diminished in IR (-56%), as well as in IGT and normotensive and hypertensive diabetic patients (-85%, -91%, and -120%, respectively). Total vasodilator capacity was similar in normoglycemic individuals (IS, IR, and IGT), whereas it was significantly decreased in normotensive (-17%) and diabetic, hypertensive (-34%) patients (Prior et al 2005).

The phospho-Akt (Thr308) level in arteries from diabetic patients was reduced to about one-half of the level in non diabetic patients, suggesting impaired insulin signaling in human diabetic vascular tissue. Augmented vasoconstriction was observed in diabetic arteries, due in part to deficiency of basal and stimulated NO production. This correlated with decreased endothelial NOS expression and activity in diabetic vessels (Okon et al 2005). The evaluation of enzymes implicated in the evolution of endothelial dysfunction associated with type 2 diabetes [lipoprotein-associated phospholipase A2, meloperoxidase (MPO) and paraoxonase (PON) activities] , may improve early diagnosis of CVD in asymptomatic patients with type 2 diabetes and can help to evaluate accelerated atherosclerosis and microvascular disease (Moldoveanu et al 2006). A study recently, using high-resolution ultrasound, measured brachial artery responses to flow-mediated vasodilatation; (endothelium-dependent vasodilatation) and nitroglycerine-induced vasodilatation; (endothelium-independent vasodilatation), compares non-traditional risk factors, such as endothelial function, plasma levels of CRP and adiponectin were measured by ELISA in Type 2 diabetic and non-diabetic patients following acute myocardial infarction. Twenty Type 2 diabetic patients were compared with 25 non-diabetic patients at baseline (1-3 days from the onset of chest pain) and at 60 days follow-up after an AMI. At 60 days follow-up, there were significant differences in FMD (1.5 compared with 4.1%; $P < 0.02$), CRP (4.23 compared with 1.46 mg/ml; $P < 0.01$) and adiponectin (3.3 compared with 5.3 ng/ml; $P < 0.05$) levels between Type 2 diabetic and non-diabetic patients. These findings may, in part explain, the poor outcome in coronary artery disease seen in Type 2 diabetes (Nystrom et al 2005). Type 2 diabetes is independently associated with impaired flow mediated dilatation (FMD). Hyperglycemia and hyperinsulinemia contribute minimally to this association. Impaired FMD may therefore, in part, explains the increased

cardiovascular disease risk in DM2, whereas the normal FMD in impaired glucose metabolism (IGM) suggests that other forms of endothelial dysfunction are important in explaining the increased cardiovascular disease risk in IGM (Henry et al 2004). On the other side, acute hyperinsulinaemia, both with and without concomitant hyperglycaemia, does not increase skin microvascular permeability, haemodynamics or parameters of endothelial dysfunction in Type 2 diabetic patients (Oomen et al 2004).

Patients with diabetes or hypertension have elevated ET-1 levels, but do not exhibit positive correlations between ET-1 levels and blood pressure, which was observed in healthy controls. Increased ET-1 levels do not induce hypertension in diabetes, but were lower in diabetic patients taking ACE inhibitors compared to those without ACE inhibitors. There is no significant association between ET-1 levels and vascular complications. These findings suggest that the plasma ET-1 level is not a marker of endothelial dysfunction but changes in plasma ET-1 levels may precede vascular complications associated with hypertension and diabetes (Schneider et al 2002). The same author studied plasma endothelin-1 (ET-1) levels in patients with diabetes mellitus or hypertension with healthy controls, and investigates whether ET-1 levels are correlated with glycemic control, metabolic parameters and vascular complications on 103 patients with type 1 diabetes, 124 patients with type 2 diabetes, 35 hypertensive patients without diabetes mellitus and 99 controls. Patients with diabetes or hypertension have elevated ET-1 levels, but do not exhibit positive correlations between ET-1 levels and BP, which was observed in healthy controls. Increased ET-1 levels do not induce hypertension in diabetes, but were lower in diabetic patients taking ACE inhibitors compared to those without ACE inhibitors. There is no significant association between ET-1 levels and vascular complications (Schneider et al 2005). Furthermore the activity of endogenous ET-1 on ET (A) receptors is enhanced in the resistance vessels of patients with diabetes, whereas their sensitivity to exogenous ET-1 is blunted (Cardillo et al 2002).

Well-controlled type 2 diabetic patients free of clinical macrovascular complications have elevated plasma markers of cardiovascular risk without having increased intima-media thickness (IMT). The elevation of plasma markers of endothelial cell activation (sE-selectin and s-ICAM-1) or inflammation (CRP) and oxidative stress (8-isoprostane) in diabetics vs. controls is distinct from and cannot be explained simply by differences in the burden of atherosclerosis as assessed by carotid intima-media thickness (Moussavi et al 2004). Activation of poly (ADP-ribose) polymerase (PARP)

is an important factor in the pathogenesis of endothelial dysfunction in diabetes. Destruction of islet cells with streptozotocin in mice induced hyperglycemia, intravascular oxidant production, DNA strand breakage, PARP activation and a selective loss of endothelium-dependent vasodilation. Treatment with a novel potent PARP inhibitor, starting after the time of islet destruction, maintained normal vascular responsiveness, despite the persistence of severe hyperglycemia (Garcia Soriano et al 2001).

Possible available therapy

Several therapeutic interventions have been tested in clinical trials aimed at improving endothelial function in patients with diabetes (Table 2). Insulin sensitizers may have a beneficial effect in the short term, but the virtual absence of trials with cardiovascular end-points precludes any definitive conclusion. Two trials offer optimism that treatment with angiotensin converting enzyme inhibitors may have a positive impact on the progression of atherosclerosis (O'Driscoll et al 1997, Mullen et al 1998; Prasad et al 2000; Hornig et al 2001), although widely used, the effect of hypolipidemic agents on endothelial function in diabetes is not clear (Evans et al 2000). The role of antioxidant therapy is controversial.

Chronic cigarette smoking has a deleterious effect on plasma cICAM-1 levels in young type 1 diabetic patients, which further supports the clinical importance of discouraging the initiation of smoking and promoting its cessation in people with type 1 diabetes (Zoppini et al 1999).

Children with type 1 diabetes have early endothelial dysfunction. Better folate status is associated with better endothelial function, as measured by higher FMD, higher FMD:GTN ratio, and lower thrombomodulin. Folate may therefore protect against endothelial dysfunction in children with diabetes (Wiltshire et al 2002).

Elevation in blood glucose and total glycosylated hemoglobin in diabetic animals was normalized after islet transplantation. Furthermore, islet transplantation completely restored the defective endothelium-dependent relaxation to acetylcholine in diabetic (Pieper et al 1995, 1996). Post-prandial state is accompanied by endothelial dysfunction in Type 2 diabetic patients and that insulin aspart improved endothelial function (Ceriello et al 2004). Few studies have shown the beneficial effect of insulin on endothelial dysfunction (Table 1). Long-term treatment with hydroxymethyl starch conjugated-deferoxamine had no effect on relaxation to nitroglycerin but completely prevented the impaired relaxation to acetylcholine in diabetic rats (Pieper and Siebeneich 1997).

Inhibition of rennin angiotensin pathway (Table 2A)

Angiotensin II has several pro-oxidative effects on the vasculature, decreasing NO bioavailability and resulting in vascular injury. ACE inhibitors are known to improve endothelial dysfunction, but the ability of angiotensin receptor blockers to improve endothelial dysfunction is less clear (Mancini et al 1996; Taddei et al 2002). Both drug classes consistently prevent coronary artery (particularly in the case of ACE inhibitors), stroke, and diabetic microvascular complications of nephropathy and retinopathy (McFarlane et al 2003). Inhibition of the renin-angiotensin system is associated with reduced incidence of new-onset diabetes. In the Heart Outcomes Prevention Evaluation (HOPE) study (Yusuf et al 2001), the incidence of diabetes was 32% lower in the ramipril-treated group than in the placebo group. In the Losartan Intervention for Endpoint reduction in hypertension (LIFE) study (Dahlof et al 2002), losartan was associated with 25% less new-onset diabetes compared with atenolol. The mechanisms responsible for the reduced incidence of diabetes observed during these trials are unknown, although possible mechanisms include increased plasma bradykinin levels, which improves insulin-mediated glucose uptake (Duka et al 2001), improved endothelial function, increased vascular NO activity and reduced vascular inflammation. More recent study in Chinese investigates the relationship between angiotensin converting enzyme (ACE) gene and endothelial dysfunction, concludes that, ACE DD genotype is related to endothelium-dependent arterial dilation in the early stage of type 2 diabetes mellitus and in healthy individuals (Xiang et al 2004). Which may hope for gene therapy in the future?

Thiazolidinediones (Table 2A)

Thiazolidinediones enhances insulin-mediated glucose uptake into insulin target tissues, through activation of peroxisome proliferator-activated receptor (PPAR) (Mudaliar et al 2001). They have direct effects on adipose tissue by suppression TNF and, possibly, leptin expression; suppress lipolysis and thus decrease plasma free fatty acid concentrations and increase plasma adiponectin levels (Hauner et al 2001, 2002) and exert direct effects on insulin-mediated glucose transport in skeletal muscle and the heart (Bishop-Bailey et al 2000; Loviscach et al 2000).

Thiazolidinedione administration reverses insulin resistance and many components of the metabolic syndrome. Treatment is generally associated with increased HDL cholesterol levels; decreased blood pressure, plasma triglyceride levels, small dense LDL cholesterol particles, PAI-1 levels and albumin

excretion rates; in addition to decreased glucose levels and reduced hemoglobin A1C levels (Day 1999; Kruszynska et al 2000; Lebovitz et al 2001; Freed et al 2002).

PPAR ligands also improve endothelial function (Dandona and Aljada 2004). Several studies have demonstrated improvements in brachial artery reactivity in patients with diabetes. (Avena et al 1998; Caballero et al 2003). Troglitazone (Watanabe et al 2000) and Rosiglitazone, improves coronary artery endothelial function in patients with insulin resistances who have no traditional risk factors for atherosclerosis as well as no impaired glucose tolerance or diabetes (Quinones et al 2002). With an increase in insulin sensitivity and a drop in fasting insulin and free fatty acid levels, thiazolidinediones in combination with hormone therapy (HT) in postmenopausal women is, however, have shown in study to attenuates endothelial function (Honisett et al 2004).

The mechanisms by which thiazolidinediones improve endothelial-dependent blood flow are unknown, but likely involve several effects. First, as described above; it has an important anti-inflammatory effects that involve decreasing circulating adipokines levels (eg, TNF, PAI-1, leptin), which are reflected by reduced high-sensitivity C-reactive protein levels; increasing adiponectin levels; decreasing vascular expression of adhesion molecules (Wakino et al 2002). Second, insulin is a vasodilator stimulating expression of eNOS through the phosphatidylinositol 3-kinase (PI3K) pathway (Zeng et al 2000). This effect of insulin is blunted in patients with insulin resistance (Kuboki et al 2000). PPAR- is expressed in endothelial cells, and its ligands have been reported to enhance NO production, possibly by stimulating the PI3K pathway and hence expression of eNOS (Kim et al 2002). Third, PPAR- ligands improve several components of the metabolic syndrome that could adversely affect endothelial function, including low HDL cholesterol levels, high triglyceride and free fatty acid levels, hypertension and carbohydrate intolerance. PPAR- ligands also decrease oxidative stress and thus are able to improve the vascular balance between NO and vasoconstrictors (Bagi et al 2004). Rosiglitazone administration for 12 weeks was shown to improve insulin sensitivity and decrease asymmetric dimethyl-arginine levels. An endogenous inhibitor of NOS, is associated with reduced NO-mediated vasodilation and enhanced adherence of mononuclear cells to the endothelium (Chan et al 2000; Stuhlinger et al 2002). In recently performed a double-blind crossover trial of 12 patients with recently diagnosed type 2 diabetes concludes that insulin resistance is a major contributor toward endothelial dysfunction in type 2 diabetes; both endothelial dysfunction and insulin resistance are amenable to treatment by rosiglitazone (Pistrosch et al 2004). Recently

studies have shown that Rosiglitazone ameliorated glomerular hyperfiltration in early type 2 diabetes, improved NO bioavailability and lessened renal end-organ damage in type 2 diabetes with microalbuminuria (Pistrosch et al 2005) and Pioglitazone improves endothelial dysfunction independently from the

observed benefits on insulin sensitivity and beta-cell function in patients with newly diagnosed type 2 diabetes and CAD, and may exert additional effects on endothelial function beyond metabolic control (Forst et al 2005; Sourij et al 2006). Furthermore the GATE study may provide the rationale and impetus

Table 2A Role of various modalities of therapy on endothelial dysfunction

| Reference | Treatment | Patient | Result on endothelial function |
|--|--|---|---|
| Role of ACEI and ARBs | | | |
| Mancini et al 1996 O'Driscoll et al 1997,1999 Mullen et al 1998 Anderson et al 2000 Prasad et al 2000 Hornig et al 2001 | ACEI (most studies) | NIDDM, CAD | +EDVD |
| Cheetham et al 2000 Hornig et al 2001 Hermann et al 2006 | ARBs | NIDDM, CAD | Debated results |
| Sato et al 2003 | Quinapril 2 /12 Tx candesartan(4–12 mg-day) | 24 pt. NIDDM, 12 wks treatment | ↑ insulin-stimulated endothelial function , ↑ vascular adiponectin gene expression clinically improves oxidant stress |
| Chaturvedi et al 2001 | lisinopril | IDDM | Circulating plasma VEGF concentration is not strongly correlated with risk factor status or microvascular disease |
| Cheetham et al 2001 | 50 mg of losartan daily for 4 wks | NIDDM | ↑ NO-mediated dilation in the conduit vessels |
| Pieper et al 2000 Arcaro et al 1999 | temocapril Captopril 25 mg tid | Diabetic rat IDDM | +EDVD +EDVD in the femoral artery of normotensive microalbuminuric Pt. |
| O'Driscoll et al 1999 | enalapril (10 mg twice daily / 4 wks | NIDDM | ↑ stimulated and basal NO-dependent endothelial function. |
| McFarlane et al 1999 Gasic et al 1999 | perindopril 4 for 12 wk Fosinopril (10 mg/ day) for 12 wks | IDDM 11 microalbuminuric NIDDM pt. | improve arterial endothelial function ↓ cVCAM-1 levels and ↓ microalbuminuria |
| Nielsen et al 1997 | lisinopril 10–20 mg dialy for 12/12 | 43 hypertensive NIDDM pts | reno- and vasculoprotective properties in hypertensive NIDDM nephropathy pts. |
| Bijlstra et al 1995 | perindopril 4–8 mg OD/ 6 /12 | 10 pt. NIDDM | +EDVD |
| Role of glitazones and other oral hypoglycemic agents | | | |
| Sourij et al 2006 | pioglitazone (30 mg/day for 12 wks | NIDDM | +EDVD |
| Caballero et al 2003 | trogliatone treatment for 12 wks | NIDDM | +EDVD |
| Forst et al 2005 (Pistrosch et al 2005) | pioglitazone Rosiglitazone for 12 wks | 179 pt. NIDDM 19 pt. NIDDM with. microalbuminuria | +EDVD ↓ glomerular hyperfiltration and NO Bioavailability and ↓end-organ damage |
| Hubacek et al 2004 Pistrosch et al 2004 Tack et al 1998 | Rotiglitazone | NIDDM | +EDVD |
| Manzella et al 2005) | repaglinide (1 mg BID) for 4/12 | 16 pt. NIDDM | improves brachial reactivity and ↓ oxidative stress indexes. |
| Bengel et al 2005 | nateglinide 120 mg t.i.d. 16 wks | 47 pt. NIDDM | No effect myocardial blood flow |
| De Mattia et al 2003 | Gliclazide for 12 wk | 15 pt NIDDM | improves both antioxidant status and NO-mediated vasodilation |
| Katakam et al 2000 Mather et al 2001 | Metformin | NIDDM | +EDVD |

for the aggressive treatment of insulin-resistant patients with glitazone therapy (Hubacek et al 2004).

Other antidiabetic

Repaglinide administration, through good control of postprandial glucose levels, improves brachial reactivity and declines oxidative stress indexes (Manzella et al 2005). While Nateglinide an oral antidiabetic insulinotropic agent neither improved nor impaired myocardial blood flow in Type 2 diabetic patients (Bengel et al 2005). Metformin; an antidiabetic agent that improves insulin sensitivity, treatment improved both insulin resistance and endothelial function, in animal and human (Katakam et al 2000; Mather et al 2001).

Statin and endothelial dysfunction (Table 2B)

Several clinical trials have demonstrated that statin treatment not only reduces serum cholesterol levels in hypercholesterolemic patients, but also substantially decreases the risk

of cardiovascular disease (Shepherd et al 1995). In current clinical use, statins can reduce LDL cholesterol levels by an average of 20%–35%, with a corresponding 30%–35% reduction in major cardiovascular outcomes. Decreases in serum cholesterol levels could account for the observed risk reduction, since LDL cholesterol has a strong, well-documented association with cardiovascular risk, and since plasma LDL apheresis has been shown to improve both endothelium-dependent vasodilation and cardiovascular risk in hypercholesterolemic patients (Thompson et al 1995; Tamai et al 1997). Several studies, however, have shown that improvements in endothelial function can occur before reductions in serum cholesterol levels. For example, cerivastatin has been shown to improve endothelial function in elderly diabetic patients within 3 days, while, even more strikingly, healthy normocholesterolemic subjects have demonstrated markedly improved endothelial vasoreactivity after 3 hours (Tsunekawa et al 2001; Omori et al 2002). These acute effects are in agreement with other studies that have reported improvements in endothelial function after

Table 2B Role of various modalities of therapy on endothelial dysfunction

| Reference | Treatment | Patient | Result on endothelial function |
|--|---|---|---|
| Role of statin and other lipid lowering agent | | | |
| Fegan et al 2005 | cerivastatin | 11 pt. NIDDM | improvement in microvascular endothelial function |
| Beishuizen et al 2005 | 0.4 mg cerivastatin | 250 pt. NIDDM | no effect on FMD in type 2 diabetes |
| Joyce et al 2004 | Pravastatin, 40 mg per day //1/12 | 9 pt. IDDM | Improve (FMD) |
| Dalla Nora et al 2003 | Atorvastatin for 1 year | 25 pt. NIDDM | Improve (FMD) |
| van Venrooij et al 2002 | 30 wks' Tx of atorvastatin 10 mg & 80 mg | 133 pt. NIDDM | Did not reverse endothelial dysfunction. |
| van Etten et al 2002 | 4 weeks of 80 mg atorvastatin daily | 23 pt. NIDDM | no effect on NO availability in forearm resistance arteries |
| Tan, Chow, Tam et al 2002 | atorvastatin (10 mg daily for 3/12, followed by 20 mg daily for 3/12) | 80 pt. NIDDM | +EDVD,(significant) |
| van de Ree et al 2001 | 6-week Tx with simvastatin 40 mg/daily | 17 pt. NIDDM | No effect |
| Dumont et al 2001 | Pravastatin (20 mg/kg/day) for 2 wks | Experimental rats | restores endothelial function |
| Tsunekawa et al 2001 | cerivastatin (0.15 mg/d) for 3 days | 27 elderly NIDDM | improved impaired endothelial function without affecting lipid profiles |
| Avogaro et al 2001 | Gemfibrozil 600 mg b.i.d, for 12 wks | 10 pt. NIDDM | improves both insulin action and FMD |
| Evans et al 2000 | Ciprofibrate 3/12 | NIDDM | improves fasting and postprandial endothelial function |
| Role of insulin | | | |
| Vehkavaara et al 2000, 2004 | insulin glargine and metformin | 49 in vivo endothelial function tests in 11 pt. NIDDM | +EDVD and endothelium-independent vasodilatation |
| Gaenger et al 2002 | insulin and metformin | 21 poorly controlled NIDDM | beneficial effects on vascular function, resulting in enhanced EDD |
| Evans et al 2003 | 6 weeks of insulin lispro (0.2 lu kg-1) and vitamin C 1-g daily | 20 pt. NIDDM | Improve endothelial dysfunction |

statin administration that do not correlate with reductions in serum LDL cholesterol levels (Kureishi et al 2000). Statins decrease high-sensitivity C-reactive protein levels by 30%–40%, independent of their cholesterol lowering capacities (Ansell et al 2003). Recent evidence also suggests that statins can improve insulin sensitivity. Cerivastatin has

been shown to improve insulin sensitivity, as determined by hyperinsulinemic-euglycemic clamp, in patients with type 2 diabetes after 3 months of treatment (Paniagua et al 2002). A similar study, in which patients with impaired fasting glucose levels were treated with atorvastatin for 16 weeks, noted a trend towards improved insulin sensitivity in the

Table 2C Role of various modalities of therapy on endothelial dysfunction

| Reference | Treatment | Patient | Result on endothelial function |
|---|---|--|---|
| Role of L-arginine | | | |
| Sahach et al 2005 Kabat et al 2006 | L-arginine | Porcine endothelial aortic cells (experimental) | Prevent endothelial dysfunction |
| De Cobelli et al 2004 Regensteiner et al 2003 | L-arginine 1-week Tx of oral L-arginine (9 g daily) or vitamins E (1800 mg) and C (1000 mg) | uremic IDDM pt. 10 premenopausal women with NIDDM function | induced vasodilatation of renal vls improved measures of endothelial |
| Role of antioxidants | | | |
| Qian et al 2006 | Injection of IL-2 (5000 and 50,000 U/ kg/ d s.c.) for 5 wks | diabetic rats | significantly ameliorated the endothelial dysfunction induced by hyperglycemia |
| Shemyakin et al 2006 | A 60 minute intraarterial infusion of the ET(A) receptor antagonist BQ123 (10 nmol/min) combined with the ET(B) receptor antagonist BQ788 (5 nmol/min) | 12 individuals with insulin resistance with no any history of diabetes | enhances EDV in subjects with insulin resistance |
| Zoukourian et al 1996 | iloprost | Diabetic erythrocyte-endothelium | Alter endothelial dysfunction |
| Clemens et al 1999 Chowienzyk et al 2000 | 200 microg octreotide/day for 6/12 raxofelast (600 mg twice daily) for 1 wk. | 27 patients (IDDM). 10 pt. NIDDM | Reduce endothelial dysfunction ↓ oxidative stress improves endothelial function |
| Bilsborough et al 2002 | pentoxifylline 400 mg tid for 8 wks | 13 pt. NIDDM | No effect on endothelial dysfunction |
| Role of vitamins | | | |
| MacKenzie et al 2006 | folate (5 mg daily) & vitamin B6 (100 mg daily) | 124 children, IDDM | +EDVD in children with IDDM. |
| McSorley et al 2005 | vitamin E | 14 pt. NIDDM | no effect |
| Pena et al 2004 Dhein et al 2003 | oral folic acid (5 mg/d) and 8 wks vitamin E | 36 subjects IDDM Diabetic rat | improves endothelial function prevent partially hyperglycemia-induced endothelial dysfunction |
| Skyrme-Jones et al 2000 Darko et al 2002 Skyrme-Jones et al 2001 Heitzer et al 2000 Skyrme-Jones et al 2001 Pinkney et al 1999 | Vit E vitamin C 1.5 g daily in 3 doses for 3 wks 1000 IU vitamin E for 3/12 tetrahydrobiopterin (500 microg/min) vitamin E supplement (1,000 IU for 3/12) vitamin E 500 U/day 3/12 | NIDDM 35 pt. NIDDM IDDM 23 pt. NIDDM IDDM 46 pt. IDDM | +EDVD No effect No effect VCAM-I and P-selectin improves endothelial function Improves (EVF) enhance FMD |
| Others | | | |
| Pieper et al 1997 | 50 mg/kg hydroxyethyl starch conjugated-deferoxamine for a total of 8 wks. | Diabetic rat | prevent diabetes-induced defects in endothelium-dependent relaxation. |
| Fuchsjager-Mayrl et al 2002 | aerobic exercise training | 26 pt. IDDM | improve endothelial function in different vascular bed |
| Pieper et al 1996 Maiorana et al 2001 Shai et al 2004 | Pancreatic cell transplantation combined aerobic & resistance exercise Moderate alcohol intake | Diabetic rat 16 pt. NIDDM 726 pt. NIDDM (Health Professionals Follow-up Study) | +EDVD Improves endothelial vasodilator function ↓ inflammation markers and endothelial dysfunction |

Abbreviations: NIDDM, non insulin dependent diabetes mellitus; IDDM, insulin dependant diabetes mellitus; FMD, flow mediated dilatation; EDVD, endothelial dependant vasodilatation; ACEI, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers, FMD, flow mediated dilatation; ET(A), endothelin(A), endothelial vasodilator function (EVF).

treatment group, as determined by oral glucose tolerance test, but this trend failed to reach significance (Costa et al 2003). Statin treatment did not, however, reduce the incidence of diabetes in at least one large prospective trial that addressed this question (Keech et al 2003).

Insulin therapy is it of help? (Table 2B)

Low and high physiological hyperinsulinemia abolished endothelium-dependent vasodilation, whereas endothelium-independent vasodilation was unaffected. Vitamin C fully restored insulin-impaired endothelial function without affecting endothelium-independent vasodilation (Arcaro 2002). Other investigators concluded that insulin therapy partly restores insulin-stimulated endothelial function in patients with type 2 diabetes and ischemic heart disease (Rask et al 2001). Another studies investigate long-term effects of insulin glargine on vascular function in patients with type 2 diabetes, the result supports the idea that long-term insulin therapy has beneficial rather than harmful effects on vascular function in type 2 diabetes (Paolisso and Giugliano 1996; Vehkavaara et al 2001).

Other modalities of therapy (Table 2C)

Few sporadic studies has raised the Role of vitamins, the benefit of L-arginine , antioxidant , and hydroxyethyl starch conjugated-deferoxamine aerobic exercise training and Pancreatic cell transplantation, the benefit which need to clarified in more extensive studies .

Where we are from endothelial dysfunction in diabetes mellitus?

We think that we still far from the core pathological process in endothelial dysfunction, extensive research together with randomized trial are needed in this entity especially in molecular biology and genetic engineering for more exploration of hidden aspect of iceberg. The future will witness increasing interest in finding reliable methods of testing endothelial function. As the measures of endothelial dysfunction become clinically applicable, this may translate into improved methods of risk assessment that help in predicting, preventing and treating cardiovascular disease. Inflammatory markers, such as CRP, will probably find their way into risk assessment; several therapeutic strategies aimed at improving endothelial function in diabetes mellitus states are under investigation. The future may holds great promise.

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