

## ENDOTHELIAL DYSFUNCTION AND TYPE 2 DIABETES

### Part 2: altered endothelial function and the effects of treatments in type 2 diabetes mellitus

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**SUMMARY** - Coronary artery, cerebrovascular and peripheral vascular disease, are the principal causes of morbidity and mortality in type 2 diabetes mellitus. The accelerated macrovascular disease in type 2 diabetes mellitus is due partly to the increased incidence of cardiovascular risk factors, such as hypertension, obesity and dyslipidemia. Advanced glycation end products, glycoxidised and oxidized low-density lipoproteins and reactive oxygen species linked to hyperglycemia have all been identified in type 2 diabetes mellitus and could accelerate macroangiopathy. Hence, the resistance to insulin is an additional independent risk factor, in association with oxidant stress, dyslipidemias, and prothrombic/hypofibrinolytic states.

The endothelium is a major organ involved by cardiovascular risk factors, such as hypercholesterolemia, hypertension, inflammation, ageing, postmenopausal status, and smoking. Changes in endothelium function may lead to the coronary artery circulation being unable to cope with the increased metabolism of myocardial muscle independently of a reduced coronary artery diameter. The way endothelial function is altered in diabetic patients is not yet fully understood, but the loss of normal endothelial function could be involved in the pathogenesis of diabetic angiopathy, as endothelial dysfunction is associated with diabetic microangiopathy and macroangiopathy. Finally, recent reports indicate that an improved metabolic control in diabetic patients, whatever the treatment used, is associated with near normalization or restoration of normal endothelial function.


**Key-words:** type 2 diabetes mellitus, diabetic angiopathy, endothelium, endothelial function, hyperglycemia, oxidant stress, hyperlipidemia, insulin, insulin resistance.

**RÉSUMÉ** - Dysfonction endothéliale et diabète de type 2. Partie 2: Altération de la fonction endothéliale et effets des traitements au cours du diabète de type 2.

Les maladies cardiovasculaires constituent la cause principale de morbidité et de mortalité au cours du diabète de type 2. La raison de cette macroangiopathie accélérée chez les diabétiques de type 2 est expliquée en partie par l'incidence accrue d'hypertension artérielle, d'obésité et de dyslipidémies, facteurs de risque cardiovasculaire. Le processus de glycation non-enzymatique des protéines, l'oxydation et la gluco-oxydation des lipoprotéines plasmatiques, ainsi que l'hyperproduction d'espèces oxygénées réactives largement décrits au cours du diabète de type 2 sont liés à l'hyperglycémie et interviennent dans le développement de la macroangiopathie. Le syndrome d'insulinorésistance pourrait constituer un facteur de risque indépendant supplémentaire, et est associé au stress oxydatif, aux dyslipidémies, et aux phénomènes prothrombotiques et/ou hypofibrinolytiques.

Il est clairement établi que l'activité des cellules endothéliales est altérée au cours de l'hypercholestérolémie, l'hypertension artérielle, les phénomènes inflammatoires, pendant la période ménopausique et chez les sujets âgés et les fumeurs. Les modifications de l'endothélium peuvent conduire à une inadaptation de la circulation coronaire liée à un métabolisme accru du muscle cardiaque indépendamment de toute réduction du diamètre de la lumière artérielle. Les mécanismes en cause dans la dysfonction endothéliale observée au cours du diabète restent à élucider, mais la perte de la fonction normale de l'endothélium pourrait être impliquée dans le développement de l'angiopathie diabétique, car elle est présente chez les patients atteints de micro- et macroangiopathie diabétiques. Enfin, plusieurs auteurs ont récemment démontré que la prise en charge du déséquilibre métabolique du patient diabétique de type 2 s'accompagne, quel que soit le traitement mis en place, d'une amélioration, voire d'une normalisation de la fonction endothéliale.

**Mots-clés :** diabète de type 2, angiopathie diabétique, endothélium, fonction endothéliale, hyperglycémie, stress oxydant, hyperlipidémie, insuline, insulinorésistance.

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**T**ype 2 diabetes mellitus is associated with an increased incidence of micro- and macroangiopathy, related to the degree of hyperglycemia and hypertension. These disorders may, together with other risk factors, such as hyperlipidemia, oxidant stress and insulin resistance state, alter endothelial function and consequently trigger the start of atherosclerosis in type 2 diabetes mellitus.

The first studies on abnormal nitric oxide (NO) production were performed in diabetic rats, and then confirmed in diabetic humans. The relaxation of coro-

nary arteries in response to pharmacological stimuli is reduced or suppressed in diabetic rats [1]. In human, the vasodilation of coronary arteries is also altered after pharmacological (acetylcholine) or mechanical (cold test) stimuli, but these abnormalities of large vessels are not associated with angiographic lesions, and are independent of other cardiovascular risk factors [2], suggesting impaired endothelium function without any anatomical lesions.

The response of endothelium-dependent vasodilation is already altered in patients with impaired fasting glycemia [3] or impaired glucose tolerance [4], and endothelial dysfunction has been reported even in uncomplicated type 2 diabetic patients [5]; it is independent of obesity in these patients [6]. These abnormalities also occur in type 2 diabetic premenopausal women when compared to healthy women [7], explaining, at least in part, the similar rates of coronary artery disease and mortality in diabetic men and women.

#### ABBREVIATIONS

<b>NO</b> :	nitric oxide
<b>NOS</b> :	nitric oxide synthase
<b>UAE</b> :	urinary albumin excretion
<b>vWF</b> :	von Willebrand factor
<b>ICAM</b> :	intercellular adhesion molecule
<b>VCAM</b> :	vascular cell adhesion molecule
<b>LDL</b> :	low density lipoprotein
<b>DAG</b> :	diacylglycerol
<b>PKC</b> :	protein kinase C
<b>ET<sub>1</sub></b> :	endothelin 1
<b>VEGF</b> :	vascular endothelium growth factor
<b>EGF</b> :	epidermal growth factor
<b>TGF-β</b> :	transforming growth factor β
<b>PAI-1</b> :	plasminogen activator inhibitor-1
<b>PGI<sub>2</sub></b> :	prostacyclin
<b>AGE</b> :	advanced glycation endproducts
<b>RAGE</b> :	receptors for advanced glycation endproducts
<b>IL-1</b> :	interleukin-1
<b>TNFα</b> :	tumor necrosis factor-α
<b>OS</b> :	oxidative stress
<b>ROS</b> :	reactive oxygen species
<b>O<sub>2</sub>•</b> :	superoxide
<b>H<sub>2</sub>O<sub>2</sub></b> :	hydrogen peroxide
<b>HO•</b> :	hydroxyl radical
<b>ONOO-</b> :	peroxynitrite
<b>SOD</b> :	superoxide dismutase
<b>FFA</b> :	free fatty acids
<b>LPL</b> :	lipoprotein lipase
<b>EL</b> :	endothelial lipase
<b>VLDL</b> :	very low density lipoprotein
<b>HDL</b> :	high density lipoprotein
<b>LPC</b> :	lysophosphatidylcholine
<b>TRL</b> :	triglyceride-rich lipoprotein
<b>TG</b> :	triglyceride
<b>TXA<sub>2</sub></b> :	thromboxane A <sub>2</sub>
<b>PL</b> :	phospholipid
<b>HUVEC</b> :	human umbilical vein endothelial cell
<b>NF-κB</b> :	nuclear factor-kappa B
<b>ACE</b> :	angiotensin converting enzyme
<b>L-NMMA</b> :	N <sup>G</sup> -monomethyl-L-arginine

#### ■ ENDOTHELIAL DYSFUNCTION AND DIABETIC ANGIOPATHY

Endothelial dysfunction appears to be closely associated with the development of microangiopathy in type 2 diabetes mellitus. Stehouwer *et al.* monitored 94 patients with type 2 diabetes mellitus for 36 months or more, and found that the urinary albumin excretion (UAE) rate was linked to factor VIII (Von Willebrand factor, vWF), a marker of endothelial dysfunction [8]. The raised baseline urinary albumin excretion rate in subjects with type 2 diabetes mellitus, with or without previous cardiovascular event, was associated with an increased risk of new cardiovascular events only in patients with high vWF concentrations (relative risk = 3.66, CI: 1.3-11.9). In another study, increased plasma Von Willebrand factor (vWF) concentrations were found only in microalbuminuric type 2 diabetic patients [9], whereas adhesion molecules (ICAMs and VCAMs) were already elevated and vasoreactivity was altered in normoalbuminuric diabetic patients compared with healthy subjects. Thus, endothelial dysfunction, as an early phenomenon, may explain the relationship between albuminuria and extrarenal complications, particularly the development of atherosclerosis. Subjects with type 2 diabetes mellitus have also impaired endothelium-independent vasoactive responses to glyceryl nitrate [10], which could be linked to diabetic neuropathy and the decreased reactivity of smooth muscle cells. Others have demonstrated that the abnormality of endothelial vasoreactivity is due to increased inactivation of NO, or to the decreased reactivity of vascular smooth muscle to NO [11], but also to the degeneration of the endothelium [12], muscarinic receptors dysfunction, or an increase in vasoconstrictor substances.

## ■ PHYSIOPATHOLOGY OF ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES MELLITUS

Endothelial function is more altered in type 2 diabetic patients than in type 1 diabetic patients, even though the blood glucose control may not be significantly different between them [13], suggesting that other factors are involved. A longer period of undetected blood glucose abnormalities, lipid alterations or decreased insulin sensitivity could be involved. Therefore, the initial acetylcholine-endothelium dependent dysfunction can be improved by the normalization of blood glucose control in type 1 diabetes mellitus [14], whereas it has never been observed in type 2 diabetes mellitus.

Consequently, hyperglycemia is responsible, at least in part, for endothelium dysfunction in type 2 diabetes mellitus. The subsequent influence of increased oxidant stress is debated, as is the influence of lipids and lipoproteins. Alternatively, insulin and/or insulin resistance may be involved (*Fig. 1*).

### The role of hyperglycemia

#### Clinical studies

Several reports have shown that endothelial function is impaired during both acute and chronic hyperglycemia.

Acute hyperglycemia depresses NO formation in the arterioles of the rat skeletal muscle spinotrapezius

[15]. These observations have been indirectly confirmed in humans, as hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of the brachial artery [16]. It has also been recently shown that transient hyperglycemia induced by an oral glucose load acutely impairs endothelium-dependent vasodilation, even in non-diabetic healthy subjects [17]. A recent study showed that the impaired endothelium-dependent flow-mediated dilation is closely linked to 3-hour postprandial blood glucose, but not to postprandial lipid parameters [18]. These studies suggest that acute hyperglycemia, as observed during the postprandial state in diabetic patients may have a deleterious effect on endothelial function.

As suggested by others, abnormal endothelial function is also directly associated with the degree of hyperglycemia, as defined by fasting blood glucose [3, 18] or plasma HbA1c [18]. The forearm blood flow response of healthy subjects to methacholine was significantly attenuated during a hyperglycemic clamp used to raise and maintain the blood glucose concentration at 16.7 mmol/l [19].

#### Experimental studies

Biochemical changes are activated by high blood glucose concentrations and can explain the adverse effects of hyperglycemia on the retina, lens, nerves and also on the endothelium. The main functions of endothelial cells, such as vasomotricity, thrombotic and fibrinolytic state, vascular permeability and vasculogenesis, may be all altered (*Fig. 2*).

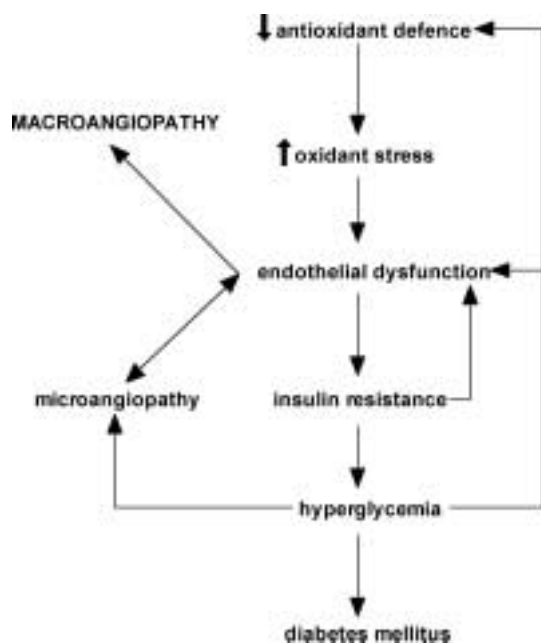


FIG. 1. Endothelial cell dysfunction and pathogenesis of diabetic angiopathy.

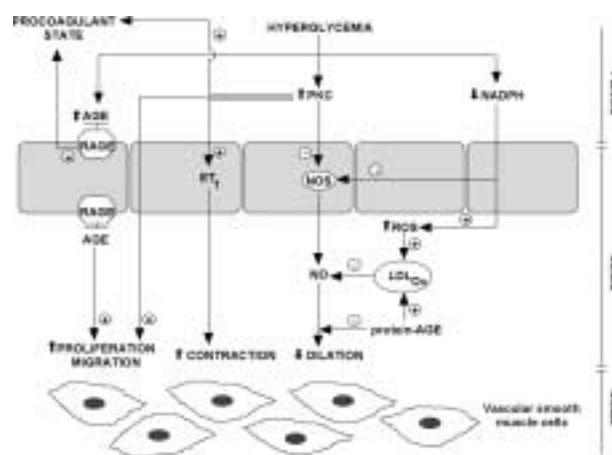


FIG. 2. The role of hyperglycemia and its metabolites in endothelial function.

NO: nitric oxide, NOS: nitric oxide synthase, ET<sub>1</sub>: endothelin-1, AGE: advanced glycation endproducts, RAGE: receptors for advanced glycation end-products, PKC: protein kinase C, LDL: low density lipoprotein, LDLox: oxidized LDL, ROS: reactive oxygen species. ○ enzyme, ◯ receptor.

In the polyol pathway, glucose is reduced into sorbitol by aldose reductase, leading to a depletion in NADPH. NADPH co-enzyme is essential for the regeneration of antioxidant molecules (reduced glutathione, ascorbate and tocopherol) and cofactor of eNOS. Sorbitol is then oxidized to fructose by sorbitol dehydrogenase enzyme. This reaction uses  $\text{NAD}^+$  and raises the  $\text{NADH}/\text{NAD}^+$  ratio modifying the redox state of the cells, and leading to the production of superoxide anions. Several studies suggest that abnormalities such as vascular permeability and flow could be due to an increase in the  $\text{NADH}/\text{NAD}^+$  ratio, directly by a decrease in  $\text{Na}^+\text{K}^+$  ATPase activity.

Hyperglycemia also increases the synthesis of diacylglycerol (DAG) by enhancing the metabolism of glucose to diacylglycerol precursors through glycolysis. This cellular metabolic regulator activates an important signal transducer, the protein kinase C (PKC) pathway. Particularly, isoform  $\beta$  is more activated in the heart and the aorta of diabetic rats. Hyperglycemia increases diacylglycerol production and protein kinase C activation, leading to a decrease in eNOS and an increase in the production of prostanoid substances by the endothelium [20]. The increased concentrations of endothelin-1 ( $\text{ET}_1$ ) in type 2 diabetes mellitus are due to the enhanced  $\text{ET}_1$  production caused by hyperglycemia, partly via activation of PKC- $\beta$  and  $\delta$  isoforms [21]. The protein kinase C increases the production of growth factors by the endothelium, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and transforming growth factor ( $\text{TGF}\beta$ ) [22]. These reactions lead to the migration and proliferation of smooth muscle cells. High circulating concentrations of plasminogen activator inhibitor-1 (PAI-1) and Von Willebrand factor have been reported in type 2 diabetic patients, together with an increase in factor X and fibrinogen activity, whereas the production of prostacyclin ( $\text{PGL}_2$ ) is reduced. Hyperglycemia, via protein kinase C activation, appears to be the most determinant of this prothrombotic and proatherogenic state.

Hyperglycemia can cause vascular dysfunction due to the interaction of advanced glycation end products (AGE) with their specific receptors (RAGE) on the endothelium. The AGE quench the NO [23] and increase the susceptibility of LDL to oxidation. The interaction and the link between AGE and their receptors leads to an increase in thrombomodulin, and also activates the receptors for the cytokines interleukin-1 (IL-1), tumor-necrosis factor- $\alpha$  ( $\text{TNF}\alpha$ ) and growth factors, leading to the migration and proliferation of smooth muscle cells.

Plasma soluble VCAM-1 levels are increased and related to hyperglycemia in type 2 diabetes [9], as are other vascular cell adhesion molecules (soluble E-selectin) [24], but the mechanisms remain unknown. The adhesion molecules VCAMs and ICAMs are increased even in uncomplicated normoalbuminu-

ric type 2 diabetic patients [9]. This altered vascular permeability facilitates the transmigration of mononuclear cells, and thus the transformation of monocytes to macrophages and then to foam cells.

### Oxidant stress

Oxidative stress (OS) is due to the production of free radicals by different metabolic pathways: increases in oxidative substrates (glucose and lipids), increased auto-oxidation, and decreased antioxidant defences. There is also a relationship between glucose auto-oxidation and non-enzymatic protein glycation, leading to the generation of free oxygen radicals (including superoxide, hydroxyl radical and hydrogen peroxide).

### Increase in oxidable substrates

Glucose oxidation leads also to the production of reactive oxygen species (ROS), such as superoxide ( $\text{O}_2\bullet$ ) via the cyclooxygenase pathway, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl radicals ( $\text{HO}\bullet$ ). Studies on the aorta of streptozotocin-induced diabetic rat showed the hyperproduction of  $\text{O}_2\bullet$  and  $\text{H}_2\text{O}_2$ , leading to the formation of hydroxyl radicals. The superoxide inhibits NO and decreases the relaxation of smooth muscle cells [25]. In diabetic humans, the production of free radicals decreases NO secretion by endothelial cells, and also inactivates NO in the sub-endothelial space [26].

Reactive oxygen species can also alter lipids and proteins, and accelerate the formation of AGE. NO rapidly reacts with  $\text{O}_2\bullet$  to form peroxynitrite ( $\text{ONOO}^-$ ), which may promote LDL oxidation.  $\text{HO}\bullet$  is responsible for the attack by radicals on phospholipid-rich cell membranes, leading to lipid peroxidation.

### Decreased antioxidant defences

Hyperglycemia also promotes glycation and inactivation of antioxidant proteins, such as Cu/Zn-superoxide dismutase (SOD), leading to its inactivation and a reduction in antioxidant defence [27]. Experimental studies in streptozotocin-induced diabetic rats have shown decreased concentrations of antioxidants like vitamin E, superoxide dismutase and catalase [28]. For example, the consumption of NADPH by the polyol pathway leads to decreased glutathione activity, which is an efficient system for capturing free radicals [29]. Experimentally *in vitro*, when the activities of superoxide dismutase (which capture  $\text{O}_2\bullet$ ) and catalase (which capture  $\text{H}_2\text{O}_2$ ) were maintained, the endothelial function was not altered even in cases of hyperglycemia.

## Roles of insulin and insulin resistance on endothelial function

### *Insulin in vivo*

Endothelial cells express insulin receptors, and insulin "*per se*" elicits NO-dependent vasodilation in human skeletal muscle [30]. Insulin can act both directly and indirectly on endothelial function. Directly, insulin itself or a second messenger in its signalling cascade stimulates basal NO production. *In vitro* evidence indicates that insulin activates the L-arginine-NO vasodilator pathway in man [31] and stimulates the release of endothelium-derived relaxing factors (NO and PGI<sub>2</sub>), leading to the relaxation of vascular smooth muscle cells. As, free fatty acids impair endothelial function [32], insulin can act indirectly by decreasing the release of free fatty acids (FFA) due to inhibition of adipocyte lipolysis.

### *Insulin resistance*

Insulin vascular action may be blunted in insulin resistant states, such as obesity [33], hypertension, impaired glucose tolerance and type 2 diabetes mellitus [6]. Insulin resistance precedes the development of type 2 diabetes mellitus and is associated with increased plasma concentrations of endothelin and vWF in obese subjects, even in the absence of diabetes mellitus [34]. Several authors have reported that acetylcholine-induced vasodilation is correlated with the insulin sensitivity in healthy subjects, suggesting that insulin plays an important role in the early processes of endothelial dysfunction [35]. Avogaro *et al.* confirm this hypothesis in uncomplicated type 2 diabetic patients with cellular glucose disposal defects linked to insulin resistance [5].

### *Endothelial dysfunction: cause or consequence of insulin resistance?*

Some authors have argued that endothelial dysfunction is not only a consequence but also a cause of most features of insulin resistance. The endothelium plays key roles in hemostasis, regulation of blood flow, maintenance of vascular architecture, and mononuclear cell transmigration, and facilitates the transport of hormones such as insulin. Deficient endothelium-dependent vasodilation may impair the postprandial increase in blood flow in insulin-sensitive tissues, considered to be determinant for glucose disposal [36]. The delay in the endothelial transport of insulin to the interstitial space may influence the action of insulin before it binds to its receptor [37]. There is a rate-limiting step in glucose disposal in response to changes in insulin concentration [38]. Endothelial dysfunction could consequently contribute to the insulin resistant states.

Some authors have developed the idea that endothelial dysfunction is the cause of not only insulin

resistance, but also of the insulin resistance syndrome [39]. Loss of endothelium-dependent vasodilation and increased synthesis of vasoconstrictors might contribute to the development of hypertension, and loss of endothelium-bound lipoprotein lipase (LPL) and/or endothelial lipase (EL) activities might create dyslipidemia, two major features of the insulin resistance syndrome. Consequently, endothelial dysfunction could precede the metabolic alterations. These demonstrations highlight the issue of vascular insensitivity to insulin, a phenomenon called "*vascular insulin resistance*".

### *Is endothelial dysfunction linked to hyperinsulinemia?*

The infusion of supra-physiological amounts of insulin decreases the endothelium-dependent vasodilation in obese Zucker rats. In the same way, high concentrations of insulin do not increase the regulation of vasoconstrictor reactivity by basal NO in the isolated aorta of the obese Zucker rat [40]. These data suggest that high insulin concentrations act on vascular signalling in this model of obesity with insulin resistance. However, it is not clear whether such effects occur at physiological insulin concentrations. The mechanisms by which hyperinsulinemia acts on endothelial function also remain to be elucidated, although it has been demonstrated that ET<sub>1</sub> is produced in response to both hyperglycemia and hyperinsulinemia [41].

### *The influence of diabetic dyslipidemia on endothelial function*

The atherogenic lipid profile in type 2 diabetes is well established, with hypertriglyceridemia associated with raised VLDL and free fatty acids, decreased circulating HDL, and particularly HDL<sub>2</sub>, and oxidized LDL. The lipoprotein lipase activity is also altered and contributes to hypertriglyceridemia, two abnormalities linked to the insulin resistant state.

### *Clinical studies*

Several authors have suggested that abnormal lipids and lipoproteins *in vivo* play a role in endothelial dysfunction in type 2 diabetes [10]. However, patients studied have often other factors that influence endothelial function. Watts *et al.* demonstrated that endothelium-dependent vasodilation was negatively and significantly correlated with elevated triglyceride concentrations, small dense LDL and low HDL cholesterol concentrations [42]. O'Brien *et al.* showed that the best predictor of endothelial dysfunction evaluated by acetylcholine perfusions to promote dilation was the low HDL-concentration [43]. Makimattila *et al.* showed that only LDL size was inversely correlated with the acetylcholine-induced brachial en-

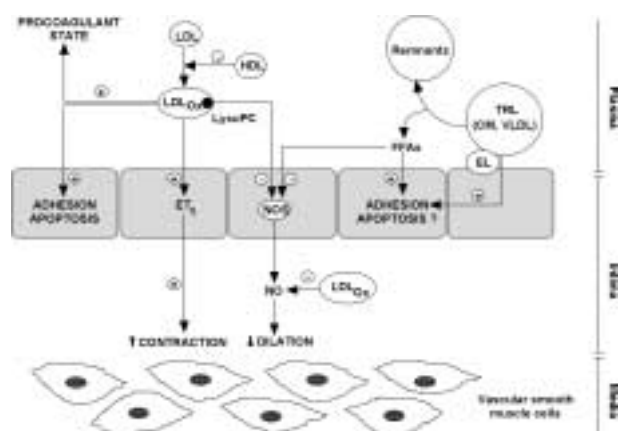


FIG. 3. The effects of lipid and lipoprotein metabolism on endothelial function.

NO: nitric oxide, NOS: nitric oxide synthase, ET<sub>1</sub>: endothelin-1, LDL: low density lipoprotein, LDLox: oxidized LDL, TRL: triglyceride-rich lipoprotein, CM: chylomicron, VLDL: very low density lipoprotein, FFA: free fatty acids, EL: endothelial lipase, HDL: high density lipoprotein, LPC: lysophosphatidylcholine.  
○ enzyme.

dothelium dependent dilation in type 2 diabetic patients [44]. And Tan *et al.* used multivariate analysis to show that the smallest LDL subfractions account for 12% of the variation in endothelium-dependent vasodilation [45]. These clinical studies together show that LDL-cholesterol is one of the major parameters involved in endothelial dysfunction.

### Experimental studies

There are few published data on how impaired endothelial function is linked to dyslipidemia in animals or humans with diabetes mellitus. However, some potential causes for the endothelial dysfunction in type 2 diabetic patients have been identified (Fig. 3).

#### LDL and oxidized LDL

The LDL particles may be modified in the diabetic state, leading to glycated LDL, oxidized LDL and glyco-oxidized LDL. Glycated LDL are more susceptible to oxidation than are native LDL. Small, dense LDL are more abundant in type 2 diabetes and are particularly prone to oxidative modifications, even when the blood glucose control is good [46].

LDLox decreases acetylcholine and serotonin-induced vasodilations in non-diabetic animals. LDLox impairs NO metabolism by inhibiting its formation and action. This could be linked to the G1 protein signal transduction pathway, by decreasing the synthesis of the protein responsible for mRNA destabilization and protein degradation [47]. LDLox may also capture the NO in the subendothelium [48], and decrease the expression of NOS mRNA and NOS activ-

ity [49]. Lysophosphatidylcholine (LPC) is a by-product of cholesterol esterification and is formed during the oxidation of LDL. Its accumulation in oxidized LDL is clearly involved in endothelium dysfunction with a negative effect on NOS [50]. Lysophosphatidylcholine can also activate protein kinase C, which in turn modulates vascular constriction and leads to the increased production of reactive oxygen species [51]. LDLox may also impair NO formation by stimulating the synthesis of caveolin-1 within caveolae [52]. The degree of endothelial dysfunction appears to be related to the time that the cells are exposed to LDLox and the concentration of these lipoproteins. This probably involves the endothelial receptor for oxidized low density lipoprotein [53]. LDLox should also enhance the release of endothelin-1, the main endothelial constrictor peptide [54].

The production of soluble adhesion molecules may be stimulated by LDLox via an exaggerated release of cytokines. *In vitro*, LDLox from type 2 diabetes can stimulate the production of radical-induced monocyte chemoattractant protein-1 mRNA in cultured human endothelial cells [55]. Incubation of human umbilical vein endothelial cells (HUVECs) with oxidized LDL, but not native LDL, leads to apoptosis of endothelial cells [56].

LDLox can modulate the PGI<sub>2</sub>/TXA<sub>2</sub> ratio, leading to a prothrombotic state. Thus, LDLox stimulates the synthesis of thrombomodulin and enhance the release of PAI-1 by cells and consequently increase the thrombotic state [57].

#### Low HDL

HDL cholesterol should hydrolyze hydrogen peroxides from the LDLox *in vitro* and then reduce the adverse effects of these lipoproteins on the endothelium. However, the impact of changes in HDL on endothelial function remains unclear. Chowieńczyk *et al.* found no alteration in NO metabolism in lipoprotein lipase deficiency patients, who typically have low plasma HDL and high triglyceride [58].

#### Triglyceride-rich lipoproteins (TRL)

Watts *et al.* have shown that the increase in TG in type 2 diabetic patients is significantly and inversely correlated with the vasodilatory response to acetylcholine [42], and hypertriglyceridemia enhances the binding of monocytes to endothelial cells [59]. An elevated TG concentration may also increase the concentrations of selectin, ICAMs and VCAMs [60], and affect the permeability and inflammation of endothelial cells. *In vitro*, the endothelium-dependent dilation of healthy rat aortic rings exposed to VLDL is impaired and strongly correlated with the phospholipid content of VLDL [61].

The triglycerides are also associated with changes in endothelial function in healthy subjects in the post-

prandial state [62]. However, the direct responsibility of triglyceride-rich lipoproteins for endothelial dysfunction during the postprandial state remains conflicting, particularly when patients with coronary artery disease are compared to healthy controls [63, 64]. Rabbit aortic responses to acetylcholine and L-NMMA *in vitro* are related to lipoproteins remnants but not native VLDL isolated from hyperlipidemic subjects [65]. The adhesion of monocytes to bovine aortic endothelial cells is also increased if the endothelium is preincubated with VLDL from fat-fed monkeys. Fard *et al.* used an endogenous inhibitor of NO synthase *in vivo* to demonstrate that the flow-mediated and the percent of brachial artery dilation of type 2 diabetic patients are inversely correlated with the increase in TG after ingestion of a high-fat meal [66]. The authors suggest that the changes in endothelial function are linked to VLDL metabolism. Others have demonstrated recently that the endothelial dysfunction of type 2 diabetic is linked to the triglyceride enrichment of VLDL and LDL during the postprandial state [67]. These experimental and clinical studies are in accordance with the discovery of a VLDL receptor in non-diabetic endothelial cells [68].

#### Free Fatty Acids

Type 2 diabetes mellitus is associated with insulin deficiency, and with insulin resistance and decreased anti-lipolytic activity of the insulin, resulting in increased free fatty acids concentrations [33]. Steinberg *et al.* have shown that elevated circulating free fatty acids concentrations blunt the endothelium-dependent vasodilation of the femoral artery [32]. The mechanisms underlying the effects of free fatty acids on endothelial cells remain debated: a decreased activity of eNOS has been reported *in vitro* [69], and others have suggested that fatty acids such as linoleic acid caused NF- $\kappa$ B-dependent transcription in cultured endothelial cells [70], leading to the synthesis of adhesion molecules.

Finally, the effects of free fatty acids on endothelial function may be strongly dependent on the ability of endothelial lipase to hydrolyse TG and phospholipids. Lipoprotein phospholipids are hydrolyzed by endothelial lipase bound to the endothelial cell surface by heparan sulfate proteoglycans, generating free fatty acids. The gene encoding this enzyme has been discovered recently [71]. This endothelial lipase has detectable triglyceride lipase activity, but this activity is significantly lower than its phospholipase activity and than is the case for hepatic lipase and especially lipoprotein lipase. The study of endothelial lipase in type 2 diabetes is very attractive considering the insulin resistant state and the relationship between lipoprotein phospholipids and endothelium-dependent relaxation [61]. The endothelial lipase enzyme seems to play a key role in modulating lipoprotein metabolism, particularly in HDL and phospholipid metabolism.

## ■ EFFECTS OF TREATMENTS ON ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES

### Blood glucose control

The control of blood glucose remains the primary goal of therapeutic regimens. However, to our knowledge, no controlled study has specifically evaluated the effects of blood glucose control on endothelium-dependent vasodilation. Jaap *et al.* showed that an enhanced hyperemic response to local heating of the foot was in good correlation with decrease in HbA1c [72]. E-selectin is lower in type 2 diabetic patients after normalization of blood glucose control with insulin. The fall in E-selectin is also correlated with the quality of blood glucose control [24]. However, Vehkavaara *et al.* found no correlation between the improvement in blood glucose control (HbA1c) and enhanced endothelial function [73]. The improvement in endothelial function with control of blood glucose probably depends on the initial hyperglycemia, as demonstrated in streptozotocin-induced diabetic rats.

### Dietary regimens

Fish oil enhances NO production by cultured human endothelial cells. Dietary supplementation with omega-3 fatty acids has beneficial effects on endothelium function in hypercholesterolemic patients *in vivo* and *in vitro* [74], but no data are available in diabetic patients. Dietary supplementation with L-arginine leads to an endothelium-dependent improvement in vasorelaxation in hypercholesterolemic patients, but its effect in diabetes remains to be investigated.

### Oral hypoglycemic agents

*In vitro* studies on mesenteric arteries showed that vascular-function is improved in metformin-treated rats, but the metformin concentrations used were over 300 mg/kg per day [75]. Marfella *et al.* showed that the changes in platelet aggregation and blood viscosity in type 2 diabetic patients in response to L-arginine (the natural precursor of NO) are significantly improved by metformin [76]. However, no data on the improvement in blood glucose control were given. The authors also reported the selective reduction in PAI-1 by metformin, confirming the data published by Nagy *et al.* [77].

Glucilazide has been shown to normalize plasma lipid peroxides, monocyte adhesion and tumor necrosis factor- $\alpha$  production in type 2 diabetes. These effects can be considered to be beneficial for endothelial function.

As acute hyperglycaemia probably impairs endothelial function, the effect of drugs that control the postprandial changes in blood glucose is attractive

with regard to endothelial function. Vallejo *et al.* showed that acarbose treatment partially improved endothelial dysfunction in streptozotocin-induced diabetic rats [78].

Tack *et al.* reported interesting data on non-diabetic insulin-resistant obese subjects treated with troglitazone. They showed that this drug improved sensitivity to insulin but had no effect on endothelium-dependent and independent vascular responses [79]. However, 200 mg daily troglitazone reduces LDL oxidation and lowers E-selectin concentration (indirect markers of endothelial function) in type 2 diabetes.

### Insulin therapy

Insulin therapy causes several changes that could enhance endothelial function, including decreases in triglyceride, free fatty acids, and glucose concentrations. Some authors suggest that the vasodilatory properties of insulin are NO dependent and insulin infusion improves the methacholine-induced endothelium-dependent vasodilation in normal subjects [30]. The impaired endothelial function in 10 non-diabetic volunteers caused by free fatty acids was also reversed by insulin infusion [80]. Insulin given at bed-time in type 2 diabetic patients together with metformin also improved endothelial-dependent dilation after 6 months of treatment, independently of blood glucose control [73].

### Hypolipidemic treatments

The beneficial effects of all hypolipidemic agents on endothelial function are due to the decrease in plasma cholesterol and LDL-cholesterol [81]. Treatment with an HMGCoA reductase inhibitor improved the reduced endothelium-dependent vasodilation in non-diabetic human [82]. Recent evidence suggests that these drugs act independently of lipid lowering. It has also been demonstrated that simvastatin preserves endothelial function even in the absence of cholesterol lowering by increasing eNOS concentrations and by decreasing oxidative stress [83], and that atorvastatin promotes NO production by decreasing caveolin-1 expression in endothelial cells [84].

Statins have beneficial effects in type 2 diabetes; they decrease plasma LDL cholesterol and increase HDL cholesterol. In a controlled trial, endothelial-dependent dilation was assessed in 21 type 2 diabetic patients with moderate hypercholesterolemia and compared to that of healthy subjects before and after 24 weeks of simvastatin treatment. Despite lowering total and LDL-cholesterol concentrations, simvastatin therapy produced no changes in endothelium-dependent or -independent brachial artery vasoreactivity [85].

By contrast, the improvement in the lipid profile of type 2 hypertriglyceridemic diabetic patients treated

with ciprofibrate was associated with improved flow-mediated endothelium-dependent dilation measured in fasting and in the postprandial state following an oral fat load [86], suggesting the influence of triglyceride-rich lipoproteins in endothelial dysfunction.

### Antioxidants and inhibitors of advanced glycation end products

Reversing oxidative stress and the subsequent inhibition of lipid peroxidation should improve endothelial function. Reaven *et al.* showed that giving type 2 diabetes patients vitamin E supplements reduced the susceptibility of LDL to copper-mediated oxidation. The effect of vitamin E on endothelial function in type 2 diabetic patients was examined by Gazis *et al.*, who found no improvement in NO-dependent vasodilation after 8 weeks of treatment [87]. Conversely, Paolisso *et al.* used 600 mg vitamin E per day in a double-blind trial and showed that 8 weeks of treatment improved endothelium-dependent dilation of the brachial artery [88].

Vitamine C is another compound that may ameliorate diabetic vascular dysfunction. The endothelium-dependent brachial vasodilation of normal subjects, which is impaired in the postprandial state and linked to TG levels, was improved by dietary supplements of the antioxidant vitamins C and E [89]. This combined effects of Vitamin C and E also prevent the endothelial dysfunction which has been observed during transient hyperglycemia after oral glucose loading in healthy subjects [17]. Acute intrabrachial administration of vitamin C reverses endothelial dysfunction in type 2 diabetic patients, but controlled clinical trials and evidence of long term benefit are lacking [90]. Finally, a new hydrophilic vitamin E-like antioxidant was given to type 2 diabetic men for 1 week and increased blood flow responses to acetylcholine [91]. Antioxidant vitamins may improve endothelial function by quenching superoxide anion (preventing NO inactivation), and preventing LDL oxidation, both of which directly inactivate NO.

Nitenberg *et al.* demonstrated that treatment with desferoxamine, an iron chelator and inhibitor of metal-catalysed hydroxyl radicals, improved the endothelium-dependent vasorelaxation in type 2 diabetic patients without coronary artery disease, but not the vasorelaxation induced by nitroglycerine [2]. Finally, Bucala *et al.* found that aminoguanidine, a nucleophilic hydrazine compound that limits the formation of AGE, improves relaxation to both acetylcholine and nitroglycerine in insulin-deficient diabetic rats under experimental conditions [23].

### Antihypertensive therapy

Hypertension contributes to endothelial dysfunction, particularly in coronary vessels. Angiotensin

converting enzyme (ACE) inhibitors have beneficial effects on hypertension, but also directly on renal function and albuminuria. In the TREND (Trial on Reversing ENdothelial Dysfunction) study, endothelial function was assessed after 6-month treatment with quinapril or a placebo in patients with coronary heart disease, including 20% diabetic patients in the treated group. Coronary endothelial function was improved in all the quinapril patients without distinction between clinical subgroups. Recently, O'Driscoll *et al.* showed that 4 weeks of enalapril treatment significantly improves endothelial-dependent function in type 2 diabetic patients [92], suggesting an early beneficial effect of angiotensin converting enzyme inhibitors in this population. However, it seems that the benefits were observed when the mean blood pressure was reduced in type 2 diabetes mellitus, whatever the drug used (angiotensin converting enzyme inhibitors or  $\beta$ -blockers) [93].

### Hormone replacement therapy

Hormone replacement with estrogens improves the endothelium-dependent vasorelaxation of coronary arteries *in vitro*. The benefits of estrogen therapy seem to be related to NO metabolism because they are attenuated by L-NMMA [94]. Hormone replacement therapy using estrogen improves endothelial function in premenopausal healthy women [95]. In contrast, medroxyprogesterone, which is commonly used in combination with estrogen, probably has a detrimental effect on endothelial responses [96].

Type 2 diabetes abrogates sex differences in endothelial function in premenopausal women [7], and this may explain the similar rates of coronary artery disease in diabetic men and women. Similarly, the recent study of Lim *et al.* showed that hormonal replacement therapy does not significantly improve microvascular reactivity in type 2 diabetic women [97].

Other molecules have been developed to improve the quality of life of post menopausal women. Raloxifene is a selective modulator of estrogen receptors (SERM) which has beneficial effects on osteoporosis in this population. This drug causes positive changes in the lipid profile, and seems to act directly on the vascular wall. Simoncini *et al.* recently showed that raloxifene stimulates NO production *in vitro* by activating NOS in HUVECs [98].

### Physical exercise

Physical training improves coronary endothelial function in non-diabetic healthy subjects [99]. Epidemiological data confirm the benefit of physical training for improving blood glucose control in type 2 diabetic patients and also for preventing the development of diabetes mellitus. Exercise may ameliorate endothelial function in type 2 diabetics, but no study has yet been done.

## CONCLUSIONS

Changes in endothelial cell function are associated with the very early stages of abnormal glucose metabolism, beginning with the impaired fasting glycaemia and impaired glucose tolerance. This correlation also occurs in hypertension and the non-diabetic obese. These findings emphasize the major role of insulin, and of insulin resistance, in the early dysfunction of endothelial cells. Chronic hyperglycemia that occurs in type 2 diabetes, and associated with hyperlipidemia and/or increased oxidative stress, are elements which both increase the initial disturbances of endothelial function. Therapeutic strategies designed at improving overall metabolic balance of type 2 diabetics are generally positive and probably complementary to the alterations of endothelial cell function in these patients. Treatments designed to reduce the cardiovascular risk factors of type 2 diabetic patients must be initiated even earlier because the alterations in the endothelium function are one of the main elements in the early development of atherosclerosis in this population.

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