Diabetologia (2001) 44: 674–692

Diabetologia

15th Golgi lecture: from hyperglycaemia to the dysregulation of vascular remodelling in diabetes

U. Di Mario, G. Pugliese

Dipartimento di Scienze Cliniche (Endocrinologia), "La Sapienza" University, Rome, Italy

Abstract

Hyperglycaemia has been shown to play a central part in diabetic vascular disease, which is also influenced by individual background. Hyperglycaemia initiates the pathogenetic sequence through a series of interrelated biochemical abnormalities, including increased flux through the polyol and hexosamine pathways, oxidative stress, AGE formation and protein kinase C activation. These abnormalities are capable of modifying the function of resident and non-resident vascular cells by changing their production pattern of several autocrine and paracrine factors, including growth, vasoactive and coagulation factors and adhesion molecules. These mediators profoundly impair the physiologic turnover of the vessel wall, thus leading to an abnormal process of vascular remodelling, with alterations in cell and matrix turnover and contacts, vascular tone and permeability and coagulation pattern. This process has distinct features depending on the target tissue. The hallmark of nephropathy is an abnormal accumulation of extracellular matrix within the mesangium, sustained by an upregulation of TGF-β, possibly triggered by a local activation of the renin-angiotensin system. The central pathological lesion in retinopathy is retinal ischaemia due to the formation of acellular capillaries. The resulting vascular endothelial growth factor-dependent neovascularization is a detrimental phenomenon leading to the formation of noncompetent vessels. Conversely, in macrovascular disease, arterial occlusion resulting from plaque formation with superimposed thrombosis elicits an angiogenic response which is impaired, but generates competent vessels, potentially compensating for reduced flow. Thus, upstream interventions interrupting the pathogenetic sequence at the level of hyperglycaemia (and related biochemical events) are the most effective, whereas downstream interventions should be targeted to the tissue affected. [Diabetologia (2001) 44: 674–692]

Keywords Vascular complications, polyol pathway, protein kinase C, nonenzymatic glycation, oxidative stress, hexosamine, cytokines, adhesion molecules, vascular remodelling, angiogenesis.

Corresponding author: U. Di Mario, MD, PhD, Dipartimento di Scienze Cliniche (Endocrinologia), Viale del Policlinico, 155, 00161 Rome, Italy

Abbreviations: ACE, angiotensin-converting enzyme; aFGF, acidic fibroblast growth factor; ANP, atrial natriuretic peptide; AR, aldose reductase; ARI, AR inhibitor; AT-II, angiotensin II; bFGF, basic fibroblast growth factor; CML, carboxymethillysine; DAG, diacylglycerol; 3-DG, 3-deoxyglucosone; DHAP, dihydroxyacetone phosphate; 1,3-DPG, 1,3-diphosphoglycerate; ET-1, endothelin-1; F-6P, fructose-6-phosphate; GA-3P, glyceraldehyde-3-phosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFAT, glucosamine-fructose-amidotransferase; GH, growth hormone; GlcN-6P, glucosamine-6-phosphate; GlcNAc-1P, N-acetylglucosamine-1-phosphate; GlcNAc-6P, N-acetylglucosamine-6-phosphate; Gly, glycerol; Gly-3P, glycerol-3-phosphate; GlyK, glycerol kinase; GO, glyoxal; GSH, reduced glutathione; GSSG, oxidised glutathione; IGFBP, IGF binding protein; MAPK, mitogen-activated protein kinase; MGO, methylglyoxal; MMP, matrix metalloproteinases; MSR, macrophage scavenger receptor; NF-kB, nuclear factor kB; O_2^-, superoxide; PA, plasminogen activator; PAI-1, plasminogen activator inhibitor-1; PDGF, platelet-derived growth factor; PG, prostaglandins; PKC, protein kinase C; RAGE, receptor for AGEs; ROS, reactive oxygen species; SD, sorbitol dehydrogenase; TX, thromboxane; UDP- GlcNAc, UDP-acetylglucosamine; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

Introduction

Diabetic vascular disease represents a major cause of mortality and morbidity in diabetic patients. Large surveys, such as the DCCT [1] and the United Kingdom Prospective Diabetes Study (UKPDS) [2], have clearly and conclusively demonstrated that hyperglycaemia plays an important part in the pathogenesis of vascular complications. However, the contribution of increased glucose concentrations to the development and progression of microvascular lesions is central, whereas several factors, in addition to hyperglycaemia, participate in the pathogenesis of macrovascular disease.

Clinical manifestations of microvascular and macrovascular involvement in diabetes include retinopathy, nephropathy and accelerated atherosclerosis, possibly leading to blindness, renal failure, myocardial infarction, stroke and limb amputation. These target organ manifestations show common pathological characteristics underlying all vascular diseases; however, each complication also has some specific features depending on the tissue(s) involved, with important implications for prevention and treatment. This diversity is supported by the distinct epidemiological characteristics of vascular complications. They occur with a different prevalence in diabetic subjects, ranging from 30 % of nephropathy to 80 % of retinopathy in patients with Type I (insulin-dependent) diabetes mellitus [3] and also according to the type of diabetes, macrovascular disease being more common in patients with Type II (non-insulin-dependent) diabetes mellitus than in those with Type I diabetes [4]. Moreover, vascular complications can be present all together or occur separately, with 17 % of diabetic patients suffering only from cardiovascular disease and 25 % from retinopathy [5–7]. Conversely, when nephropathy develops, there is usually some degree of retinopathy and cardiovascular disease present. The incidence of vascular complications also varies with the site involved. Macroangiopathy is sometimes present at the time of diagnosis, particularly in Type II diabetic patients [4], whereas microvascular disease usually develops several years after the onset of diabetes [8, 9]. However, while the incidence of retinopathy increases with diabetes duration, that of nephropathy reaches a peak after about 15 years and declines thereafter.

The question arises as to why and how hyperglycaemia produces such a diverse range of scenarios. If we go back along the pathway leading to diabetic vascular disease, we can establish that all its manifestations underlie an unregulated process of tissue remodelling, which is sustained by an abnormal expression pattern of factors regulating tissue homeostasis, occurring at the level of resident and non-resident vascular cells. This sequence of events is not specific for diabetic vascular disease, because it is also operating in other degenerative and inflammatory conditions not related to diabetes but it is specifically triggered by hyperglycaemia and the biochemical and metabolic abnormalities associated with it. The rate of development and the severity of vascular injury are also dependent on the individual background; as a result, some people show only initial lesions, whereas others progress towards end-stage disease, despite a similar degree of metabolic derangement. In this article, we will follow this track forward and review the most recent findings describing the itinerary that starts from hyperglycaemia and ends with the unregulated vascular remodelling underlying diabetic vascular disease, with particular attention to the specific features of its various manifestations.

Individual background

Both genes and the environment contribute to the impaired glucose regulation leading to hyperglycaemia, with their complex and yet undefined interaction resulting in a reduction of insulin secretion and/or sensitivity. Recently, it has been shown that although reduced insulin secretion and sensitivity both contribute to the subsequent occurrence of diabetes in the non-diabetic population, subjects with insulin resistance are at higher risk for cardiovascular disease than subjects with impaired beta-cell secretory function [10]. This seems to be confirmed by a large survey in the Japanese population indicating that the possible clinical counterpart of insulin resistance, i. e. IGT, is associated with increased cardiovascular mortality, as compared with IFG, which might be dependent on a defect of insulin secretion capacity [11].

The relation between insulin-resistance and macroangiopathy could account for the finding of established cardiovascular disease in Type II diabetic patients at the time of diagnosis [8, 9] and, possibly, the failure of tight control to prevent this complication [1,2]. In these patients, abnormalities such as dyslipidaemia and hypertension, which are part of the so-called metabolic syndrome, together with IGT or diabetes, can precede hyperglycaemia and affect vessels independently of it [12]. Reduced insulin signalling through certain transduction pathways results in impaired peripheral action, whereas stimulation from increased insulin concentrations through unaffected pathways leads to an enhanced hormonal effect. Preliminary results indicate that activation of nitric oxide synthase, an anti-atherogenic property of insulin [13], might be depressed in resistance states, whereas production of the pro-atherogenic, anti-fibrinolytic factor plasminogen activator inhibitor 1 (PAI-1) might increase in response to hyperinsulinaemia [14].

Other factors could specifically modulate the impact of hyperglycaemia on the vessel wall by either favouring the injurious effect of diabetes or protect-

ing the vasculature from it, at any level of the pathogenetic sequence. The genetic nature of this susceptibility (or resistance) has been suggested by the observation of a familiar clustering of vascular complications, which could not be explained by familiar sharing of an environmental risk factor. The importance of genetic factors has emerged predominantly for renal [15] and cardiovascular [16] disease. Genes coding for proteins involved in the regulation of blood pressure, extracellular matrix (ECM) and glucose metabolism are believed to play a role in the pathogenesis of diabetic vascular disease. Due to the conflicting results of linkage studies, none of these genes has so far been identified as being able to confer prediposition to complications; however, mounting evidence indicates that certain polymorphisms of the aldose reductase (*AR*) [17] and angiotensin-converting enzyme (*ACE*) [18] genes could be involved.

Hyperglycaemia and associated biochemical and metabolic abnormalities

The injurious effects of hyperglycaemia are characteristically observed in tissues which are not dependent on insulin for glucose entry into the cell and, hence, are not capable of down-regulating glucose transport along with the increase of extracellular sugar concentrations. Glucose uptake has been reported to be increased in mesangial cells exposed to high glucose media via upregulation of Glut-1 expression [19] and transfection of these cells with Glut-1 cDNA has been shown to mimic the effects of hyperglycaemia at normal glucose concentrations [20].

Under normal conditions, the bulk of glucose is metabolised through the glycolytic pathway and the pentose shunt. When hyperglycaemia occurs, glucose disposal through these pathways tends to increase [21]. In addition, an increased amount of glucose is converted into sorbitol, via the polyol pathway, normally operating for converting aldehydes into alcohols at physiologic glucose concentrations [22]; glucosamine-6-phosphate (GlcN-6P), via the hexosamine pathway and the glucosamine-fructose-amidotransferase (GFAT) enzyme [23]; diacylglycerol (DAG), via its de novo synthesis from glucose [24], and triglycerides, whose concentrations increase also due to the contribution of insulin deficiency and/or resistance. Part of the excess sugar reacts non-enzymatically with proteins or other circulating and tissue constituents, thus increasing the physiologic rate of non-enzymatic glycation [25]. Moreover, glucose undergoes auto-oxidation resulting, together with free radical generation from several enzymatic and nonenzymatic reactions, into oxidative stress [26].

Accumulation of intermediates of these pathways has been shown in vitro, in cell culture systems when exposed to high glucose concentrations, and in vivo, in tissues from experimental animal models of diabetes and human patients. A large body of experimental evidence has indicated that all these biochemical and metabolic consequences of hyperglycaemia are involved in mediating the injurious effects of hyperglycaemia. Reproduction of selected biochemical abnormalities has mimicked functional and structural changes observed in the tissues targets of diabetic vascular disease and the pharmacological blockade of these pathways has resulted in the prevention or retardation of vascular complications in experimental diabetic animals. These results have prompted the start of human trials using these agents. Some of these trials have already been completed, whereas others are still underway. Data available, so far, would not appear to be in agreement with results of animal studies, possibly from inadequate trial designs, in terms of number of patients and the stage of the disease at which intervention is started. In addition, the duration and dosage of the treatment used could not be sufficient for obtaining complete and persistent blockade of the target pathway within the tissue [17]. On the other hand, this finding could be related to the multifactorial nature of vascular disease; this could require the simultaneous blockade of more than one process or the identification of the central alteration and its relation with the other abnormalities. Indeed these biochemical and metabolic consequences of hyperglycaemia are not independent phenomena but, rather, they are strictly interrelated and potentiate between each other. An important corollary of this scenario is that one of these abnormalities could represent a crossroad from which all the others originate or at which they converge; hence, blocking this upstream or downstream alteration with proper inhibitors would interrupt the cascade of events triggered by hyperglycaemia.

Polyol pathway activation and the "reductive stress" hypothesis. Polyol pathway-dependent alterations in the cytosolic redox state could potentially account for increased free radical generation, AGE accumulation, protein kinase C (PKC) activation and hexosamine formation (Fig. 1). Under condition of hyperglycaemia, the flux through the polyol pathway is greatly increased and the reduction of NADPH concentrations coupled to glucose conversion into sorbitol by AR causes depletion of reduced glutathione (GSH) and oxidative stress [27]. Moreover, sorbitol oxidation to fructose by sorbitol dehydrogenase (SD) is associated with increased NADH/NAD^+ ratio, which has been referred to as "reductive stress" or "hyperglycaemic psesudohypoxia", because it mimics the redox changes observed in ischaemia [22]. The increase in NADH drives the reaction catalysed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) towards glyceraldehyde-3-phosphate (GA-3P), which is the precursor of the AGE-forming

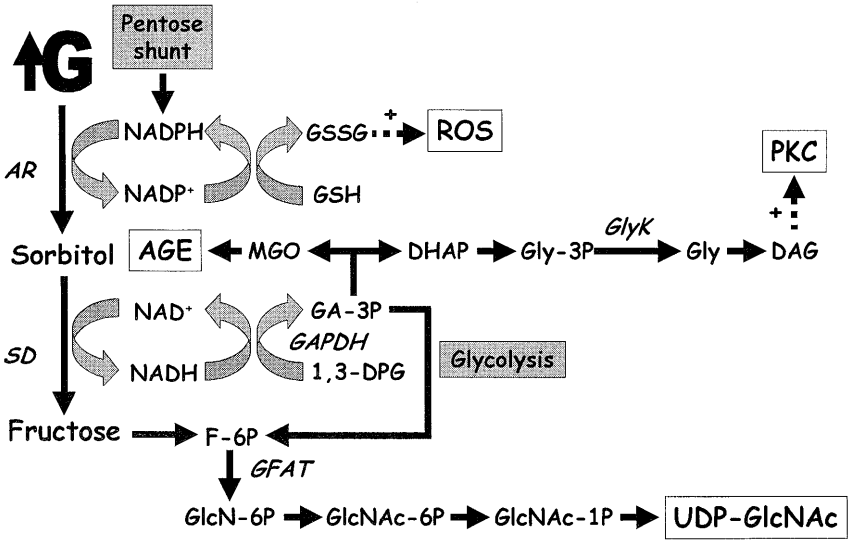

Fig. 1. Polyol-pathway-dependent induction of oxidative stress, AGE formation, PKC activation and hexosamine accumulation. A*R,* aldose reductase; *SD,* sorbitol dehydrogenase; *GAPDH,* glyceraldehyde-3-phosphate dehydrogenase; *GFAT,* glucosamine-fructose-amidotransferase; *GlyK,* glycerol kinase; 1,3-DPG, 1,3-diphosphoglycerate; GA-3P, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; Gly-3P, glycerol-3-phosphate; Gly, glycerol; DAG, diacylglycerol; PKC, protein kinase C; MGO, methylglyoxal; GSH, reduced glutathione; GSSG, oxidised glutathione; ROS, reactive oxygen species; F-6P, fructose-6-phosphate; GlcN-6P, glucosamine-6-phosphate; GlcNAc-6P, acetylglucosamine-6-phosphate; GlcNAc-1P, acetylglucosamine-1-phosphate; UDP- GlcNAc, UDP-acetylglucosamine

compound methylglyoxal (MGO) and also of the endogenous PKC activator DAG. In addition, the blockade of the glycolytic route at the level of GA-3P conversion into 1,3-diphosphoglycerate by GAPDH as well as the increased fructose formation by SD cause the activation of the hexosamine pathway with conversion of fructose-6-phosphate (F-6P) into GlcN-6P by the GFAT enzyme. The increased glucose flux through the polyol pathway is also accompanied by myoinositol depletion, altered phosphoinositide turnover, decreased calcium concentrations, and reduced Na^+/K^+-ATPase activity, which might contribute to vascular complications [21].

A major criticism to this scenario is that it seems to occur only in tissues where the activity of AR is high, such as the nerve. This is in keeping with the reported efficacy of AR inhibitors (ARIs) in preventing diabetic neuropathy in experimental animals [28] and the encouraging results obtained with these agents in initial human trials on this complication [29]. On the contrary, in vascular tissues, the low AR activity does not seem to be sufficient to reduce GSH and increase oxidised glutathione (GSSG) concentrations [30] and inhibit triose phosphate oxidation [31]. This finding is consistent with animal studies showing the failure of ARIs to prevent the development of glomerular [32, 33] and retinal [34] lesions in long-term experimental diabetes, at variance with early and advanced functional changes, such as increased renal blood flow, glomerular hyperfiltration and hypertrophy and proteinuria [35–37] as well as augmented blood flow and permeability and electrophysiologic abnormalities at the retinal level [36–38].

Oxidative stress as single unifying mechanisms. An alternative hypothesis has been recently proposed indicating oxidative stress as a single unifying mechanism linking the various biochemical pathways triggered by hyperglycaemia [39]. The initial event would be the enhanced generation of reactive oxygen species (ROS) occurring at the mitochondrial level as a consequence of the increased intracellular glucose metabolism (Fig. 2). Under these conditions, the increased proton gradient produced by the accelerated electron flow through the respiratory chain associated with excess glucose disposal is capable of generating ROS, as shown by the prevention of ROS production with an inhibitor of mitochondrial complex II or uncouplers of oxidative phosphorylation in bovine aortic endothelial cells cultured in high glucose containing media [40]. The blockade of ROS production by manganese superoxide dismutase and an inhibitor of pyruvate transport [40] indicated that the oxygen free radical produced is superoxide (O_2^-) and that the major source of it is pyruvate, which is generated by glycolysis and then transferred into the mitochondria for oxidation by the tricarboxylic acid or Krebs' cycle. Increased O_2^- production would in turn be responsible for the increased glucose flux through the polyol pathway, AGE accumulation, PKC activation and hexosamine formation produced by hyperglycaemia, together with nuclear factor kB (NF-κB) activation [39]. This was demonstrated by the prevention of increased sorbitol and MGO concentrations and PKC and hexosamine pathway activity by normalisa-

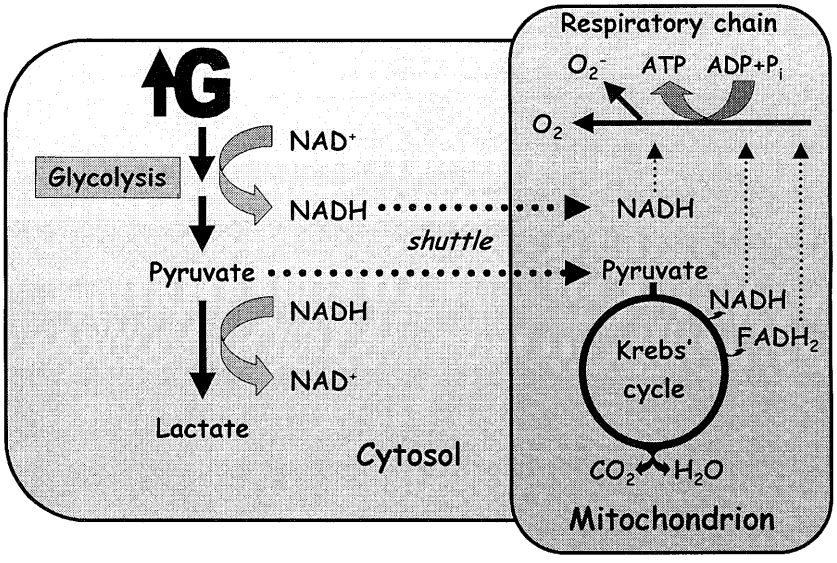

Fig. 2. Enhanced mitochondrial superoxide generation as a consequence of the increased intracellular glucose metabolism. O_2^-, superoxide

tion of mitochondrial O_2^- production in the same in vitro model [40]. The mechanism by which enhanced O_2^- concentrations increase the activity of polyol pathway is linked to its ability to quench nitric oxide, which downregulates the rate-limiting enzyme AR by modifying a cysteine residue in its active site. GAPDH inhibition by O_2^- would be responsible for the increased formation of MGO, GlcN-6P and DAG, the latter occurring also by phospholipase D activation [39].

Based on this hypothesis, the use of an antioxidant which is capable of blocking excess O_2^- production at the mitochondrial level would be the ideal approach to vascular complications of diabetes. The use of the antioxidants nitecapone [41] and D-α-tocopherol [42] was found to prevent albuminuria in rats with experimental diabetes. Antioxidants were also effective in diabetic neuropathy [43], whereas less convincing results have been forthcoming in experimental retinopathy, which was only slightly affected by antioxidant nicanartine [44]. Several studies have now been carried out in humans with the use of several different antioxidants. Controversial results have, however, been obtained [45–47], possibly because type, dosage and route of administration of these agents is critical for achieving the therapeutic goal.

One pitfall of this hypothesis could be that the electrons carried by mitochondrial NADH produced by pyruvate oxidation in the tricarboxylic acid cycle are donated to complex I, whose inhibition with rotenone failed to prevent O_2^- production [40], and not to complex II, which is fuelled by $FADH_2$. Another possible criticism is that AGEs are formed from non-oxidative reactions. Indeed, generation of MGO by fragmentation of GA-3P is favoured by oxidative stress through GAPDH inhibition, but can occur also under nonoxidative conditions, at variance with production of glyoxal, pentosidine and carboxymethillysine [48]. Moreover, if MGO is the main AGE-forming compound intracellularly, at least in endothelial cells [40], other AGE-forming compounds have been found in diabetic tissues, including deoxyglucosones deriving from non-oxidative decomposition of the Amadori product [48].

AGEs and the "carbonyl stress" hypothesis. The "carbonyl stress" hypothesis postulates that reactive carbonyl precursors of AGEs are formed from both oxidative and non-oxidative reactions from an increase of substrate availability and/or a decrease in the efficiency of detoxification systems [48] (Fig. 3). Carbonyl substrates are carbohydrates, such as glucose, fructose, triose phosphates and ascorbate, which increase in diabetes, and lipids, which are increased in dyslipidaemias, thus explaining the detection of increased circulating and vascular tissue AGE concentrations in these two conditions; also proteins can be a source of carbonyls. Even in the absence of increased substrate availability, such as in uremia, AGEs can accumulate, possibly due to increased oxidation. Another explanation for the increased AGE concentrations observed in heterogeneous pathological conditions, such as diabetes, atherosclerosis, uremia, Alzheimer's disease and dialysis-related amyloidosis, is a deficiency in (or overload on) pathways of detoxification of reactive carbonyls [48]. These pathways include the GSH-catalysed rearrangement of dicarbonyls to hydroxyacids by glycoxalase, the NADPH-dependent reduction of aldehydes to alcohols by AR and their NAD^+-dependent oxidation to carboxylic acids by aldehyde dehydrogenase [48]. Shifts in the GSH:GSSG, $NADPH:NADP^+$ and $NADH:NAD^+$

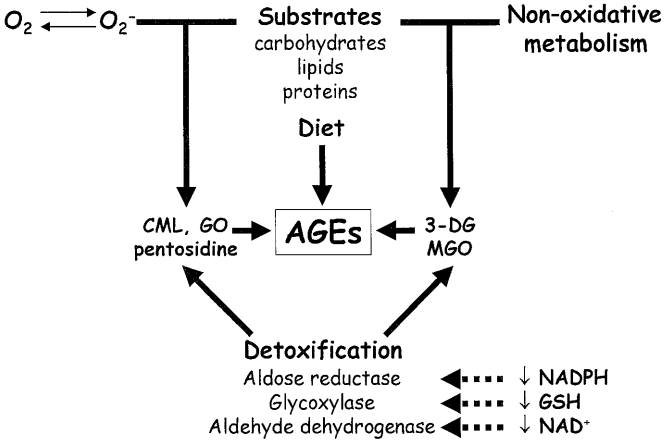

Fig. 3. Source and mechanisms of accumulation of AGEs in diabetes. O_2^-, superoxide; CML, carboxymethillysine; GO, glyoxal; 3-DG, 3- deoxyglucosone; MGO, methylglyoxal

ratios occurring in diabetes might be responsible for the impairment in the detoxification mechanisms favouring AGE formation. Aldose reductase blockade with selective inhibitors would result also in impaired detoxification of reactive carbonyls, suggesting a possible explanation for the failure of these agents to prevent microvascular lesions in long-term experimental diabetes. Based on this hypothesis, carbonyl compounds increase in diabetes as a result of both excess glucose disposal through multiple biochemical routes, which deplete cofactors for detoxification systems and provide increased amounts of substrates for non-oxidative generation of carbonyls, and oxidative stress, which favours their formation from autoxidation of glucose and early glycation products. Dyslipidaemia, frequently occurring in diabetic subjects, represents an additional source of carbonyl compounds. In addition to those forming from reaction of carbonyls with amino groups of proteins, lipids, glucoconjugates and nucleic acids, exogenous AGEs can be introduced with the diet, thus contributing to the overloading of the detoxification systems [49].

AGEs can induce both direct and indirect effects which appear to be relevant to the development of vascular lesions [50]. The direct effects include trapping of circulating macromolecules and cross-linking of irreversibly glycated tissue constituents [25]. The indirect effects of AGEs are mediated by cell surface receptors, which are coupled to signalling pathways leading to the activation of transcription factors and the modulation of gene expression; the AGE-receptor-mediated pathway is involved also in the removal of AGEs by receptor-mediated internalisation followed by intracellular degradation [50]. The use of aminoguanidine, which blocks AGE formation by interacting with the Amadori-derived fragmentation products [51], was proven to be successful in preventing experimental diabetic nephropathy [52] and vascular dysfunction [53]. Also retinopathy [54] and neuropathy [55] were shown to be favourably affected by aminoguanidine treatment. Unfortunately, human trials using this agent have not been successful [56] perhaps because aminoguanidine blocks only one, likely minor, route of AGE formation within the cell. Hopefully, new compounds acting at a different level, such as the AGE breakers, which are capable of interrupting crosslinks [57], might prove more effective in preventing AGE-induced tissue injury than aminoguanidine.

Another potential approach is interfering with cell receptors mediating the effects of AGEs. Several AGE-binding proteins have been identified so far, including the receptor for AGEs (RAGE), a 35 000 M_r member of the immunoglobulin superfamily of receptors [58]; the macrophage scavenger receptor (MSR)-A (particularly type 2) [59]; AGE-R1/*p60,* a 50 000 M_r protein homologous to the component of the oligosaccharyl transferase complex OST-48 [60]; AGE-R2/*p90*, a 80000 M_r protein homologous to the PKC substrate 80K-H [60]; and AGE-R3/galectin-3, a 32 000 M_r protein previously known as Mac-2 [61] (Table 1). This extreme heterogeneity of AGE receptors could imply binding or functional specificity or both. It is still not known, however, whether or not different AGE structures bind different receptors and whether or not these receptor molecules play distinct functional roles; alternatively, not all these receptors might be relevant to AGE binding in vivo [62]. At present, RAGE is believed to mediate AGE-induced cell activation via induction of ROS production and stimulation of p21(ras)-dependent mitogen-activated protein kinase (MAPK) and its downstream targets, NF-κB and the AP-1 complex [63,64]. In fact, the soluble fragment of RAGE and an antibody raised against this receptor were shown to suppress accelerated atherosclerosis induced by diabetes in the susceptible apoE-null mice [65] and to prevent vascular dysfunction induced by diabetic erythrocytes [66]. On the contrary, the MSR seems to also play a relevant role in vivo, being involved mainly in AGE removal, as shown by the reduced uptake of oxidised LDL by monocytes-macrophages from a null mouse carrying a mutation in the *MSR-A* gene [67]. AGE-R1/*p60*, AGE-R2/*p90*, and AGE-R3/galectin-3 interact with each other and seem to behave as an AGE-receptor complex, with the former implicated predominantly in AGE uptake and degradation and the latter two in cell activation and receptor regulation [68]. Recently, we have reported that galectin-3 is not expressed at the glomerular and/or mesangial level under normal conditions, but becomes expressed with aging, and that the diabetic milieu induces galectin-3 expression, which occurs earlier and to a greater extent than during normal aging [69]. Moreover, mice knockout for galectin-3 develop accelerated diabetic glomerulopathy associated with more marked AGE accumulation, as compared with wild-type animals [70]. These data

Table 1. AGE-binding proteins

Protein	MW	Structural features	Function
RAGE	35 000 M_r	member of the Ig superfamily proteolytic fragment of a 45 kDa protein forming complex with lactoferrin-like protein	cell activation oxidative stress
Scavenger receptor type 1	220 000 M_r	homotrimeric protein with five domains: N-terminal cytoplasmic, transmembrane, spacer of 2-N linked sites, collagen-like triple helix, C-terminal cysteine-rich	AGE uptake and degradation
Scavenger receptor type 2	170 000 M_r	as scavenger receptor I except for the cysteine-rich domain, replaced by a 6-residue C-terminus.	AGE uptake and degradation
p60 (AGE-R1)	50 000 M_r	homologous to the oligosaccharil transferase complex OST-48; forms a complex with DAD1 and ribophorin I and II in the endoplasmic reticulum.	AGE uptake and degradation
p90 (AGE-R2)	80 000 M_r	homologous to the PKC and FGFR3 substrate 80K-H; when phosphorylated binds the SH2 domain of Grb2 in a complex with Sos	cell activation
Galectin-3 (AGE-R3)	32 000 M_r	previously known as Mac-2 or carbohydrate-binding protein (CBP)-35 C-terminal CHO binding and N-terminal PGAY repeating domain	receptor regulation

RAGE = receptor for AGEs

indicate that also galectin-3 plays a substantive role as an AGE-receptor in vivo and that this AGE-receptor-mediated pathway functions as a protective mechanism towards AGE-induced tissue injury, as opposed to that through RAGE.

Recently, a growing body of evidence suggests that also Amadori products could participate in mediating cytokine and matrix changes induced by diabetes at the glomerular level, possibly via structurally distinct cell surface receptors [71]. Amadori-modified albumin was shown to induce TGF-β1 and its type 2 receptor, together with matrix proteins, in cultured mesangial [72]. Anti-glycated albumin antibodies prevented these changes [72] as well as nephropathy occurring in *db*/*db* mice [73].

PKC activation as final common pathway. Persistent activation of PKC might also operate as a final common pathway mediating tissue injury induced by hyperglycaemia and associated biochemical and metabolic abnormalities [24]. It results primarily from enhanced de novo synthesis of DAG from glucose via triose phosphates, whose availability is increased because of the enhanced glucose flux through the polyol, hexosamine, pentose and glycolytic pathways [24]. Furthermore, both the increased cytosolic NADH:-NAD^+ associated with sorbitol oxidation to fructose [21] and GADPH inhibition by ROS [38] could divert GA-3P away from the glycolytic route and towards dihydroxyacetone phosphate and DAG. Finally, recent evidence suggests that the enhanced activity of PKC enzyme could also result from the interaction between AGEs and their cell surface receptors [74].

Prevention of microvascular and neuropathic complications in diabetic animals using the selective β_2 isoform PKC inhibitor LY333531 provided experimental support to this hypothesis [75–77]; human trials are currently in progress. A potential limitation of this approach is that the PKC isoforms implicated in the increased enzyme activity differ according to the tissue target of complication. In fact, though most reports show that the β isoform is activated in vascular tissues [78–80], studies in aortic endothelial cells have indicated that the α isoenzyme might be preferentially affected at this level [81].

The hexosamine pathway and glucose toxicity. A growing body of evidence indicates that also the increased flux through the hexosamine pathway might be an important crossroad, at which all the other biochemical pathways triggered by hyperglycaemia converge to favour GFAT activity by providing increased amounts of the substrate F-6P and impairing its oxidation through the glycolytic route. Glucotoxicity by hexosamines include both the mediation of the injurious effects of hyperglycaemia [23] and the induction of insulin resistance [82], which is known to impact independently on vessels.

The GFAT-dependent increase in tissue hexosamine concentrations causes translocation of PKC α, β and ε isoenzymes, as shown in cultured mesangial cells [83]. Moreover, it induces increased O-linked glycosylation and reduced serine-threonine phosphorylation of the transcription factor Sp1 resulting in its activation with expression of the cytokines TGF-β1 and PAI-1, as shown in bovine aortic endothelial cells [84]. Studies in cultured mesangial cells indicated that TGF-β1 and matrix overproduction could be mediated by the hexosamine pathway, because the effects of high glucose were mimicked by D-glucosamine and blocked by GFAT inhibition [85]. Moreover, hexosamines feed back on glucose transport, thus reducing glucose utilisation, possibly due to altered PKC-mediated Glut-4 translocation and/or trafficking [86].

Transient vs sustained increase of blood glucose concentrations. At present, no single unifying mechanism linking all these biochemical abnormalities has been conclusively determined, though significant improvements in the knowledge of the interrelations between these pathways have been made. It is possible that each of these abnormalities or proposed sequence of biochemical events plays a different role depending on the tissue target of complication, on substrate availability, as well as on the cofactor concentration and enzyme activity. This could explain the different role of polyol pathway-dependent changes at the neural and vascular level.

Another possibility, which has recently gained consideration [87], is that the relevance of the different biochemical mechanisms might vary with the trend of increases of blood glucose throughout the day. Repeated hyperglycaemic peaks, such as those occurring postprandially or after a hypoglycaemic episode, induce acute metabolic imbalances, which rapidly reverse upon restoration of euglycemia. These changes include polyol, hexosamine and DAG pathway activation as well as oxidative stress resulting from mitochondral O_2^- generation. A sustained increase of glycaemia is associated with cumulative changes in long-lived macromolecules, which persist despite a return to normal glucose concentration. The formation of AGEs is the major mechanism involved in mediating these chronic alterations, though accumulation of some of these products within the cell can occur after a few hours and can be favoured by acute metabolic imbalances impairing the activity of detoxifying enzymes. Recently, a new mechanism has been proposed explaining the phenomenon of hyperglycaemic memory, i.e. the persistence of biochemical alteration after normalisation of glycaemia. Repeated hyperglycaemic peaks producing acute bursts of mitochondrial O_2^- production might in fact cause mutations in mitochondrial DNA, which could be responsible for the perpetuation of ROS generation by the respiratory chain at physiologic concentrations of glucose and reducing equivalents derived from its intracellular metabolism [38].

Mediators of hyperglycaemia-induced injury

Evidence indicates that hyperglycaemia and the associated biochemical and metabolic abnormalities are capable of modifying the function of resident and nonresident vascular cells by signalling through specific sensors or receptors. These cells include endothelial, mesangial and smooth muscle cells, pericytes, platelets, lymphocytes and monocyte-macrophages, change the production pattern of a series of cytokines, which, in turn, influence the function of the producing cells and those surrounding them, by acting in an autocrine and a paracrine fashion. These mediators include growth factors, vasoactive agents, coagulation factors and adhesion molecules (Fig. 4).

Several studies in experimental animal models and cell culture systems have attempted to evaluate the role of various autocrine and paracrine factors in diabetic vascular disease. To establish such a role, it would be necessary to demonstrate that (1) the local synthesis and/or action of a certain cytokine are actually increased in diabetic vascular disease; (2) the cytokine is capable of inducing functional and structural changes in the target tissue mimicking those observed in the diabetic condition; and (3) blocking the expression of the cytokine or the receptor(s) mediating its effects can prevent or attenuate the complication [88]. At present, experimental evidence indicates that several autocrine and paracrine factors could be involved in the pathogenesis of diabetic vascular disease, although no single factor has yet been identified as to the causative in the development of this complication.

High glucose concentrations and cytokine alterations. When vascular cells are exposed to high glucose concentrations, a number of cytokine alterations can be detected. Both peptide and mRNA expression of TGF-β are increased in mesangial cells grown in high glucose; under these conditions, the bioactivity of this growth factor is increased as well [89]. Concentrations of IGF-I peptide and IGF-I and II mRNA expression also increased in this in vitro model and these changes were associated with increased IGF-I receptor number and reduced IGFBP production, possibly implying an overall activation of the mesangial IGF system under experimental conditions mimicking hyperglycaemia [90]. An upregulation of vascular endothelial growth factor (VEGF) gene expression has been found in retinal endothelial cells and pericytes grown in high glucose [91].

Among vasoactive agents, nitric oxide has been shown to vary with the duration of exposure of endothelial cells to high glucose, with concentrations of nitrites-nitrates and endothelial isoform of nitric oxide synthase that are increased after short term incubation [92] and reduced upon chronic exposure to hyperglycaemic media [93]. Endothelin-1 (ET-1) was also shown to be increased by high glucose in retinal endothelial cells and also in pericytes [94]. Eicosanoid production appears to be influenced as well under hyperglycaemic conditions. Reduced concentrations of the vasodilatory prostanoid prostaglandin (PG) E_2 and unchanged concentrations of the vasoconstrictor eicosanoids thromboxane (TX) B_2, the stable metabolite of TXA_2, and $PGF_{2\alpha}$ were shown after prolonged exposure of mesangial cells to high glucose concentrations [95], whereas increased production was reported in mesangial cells incubated in high glucose for short time periods [96].

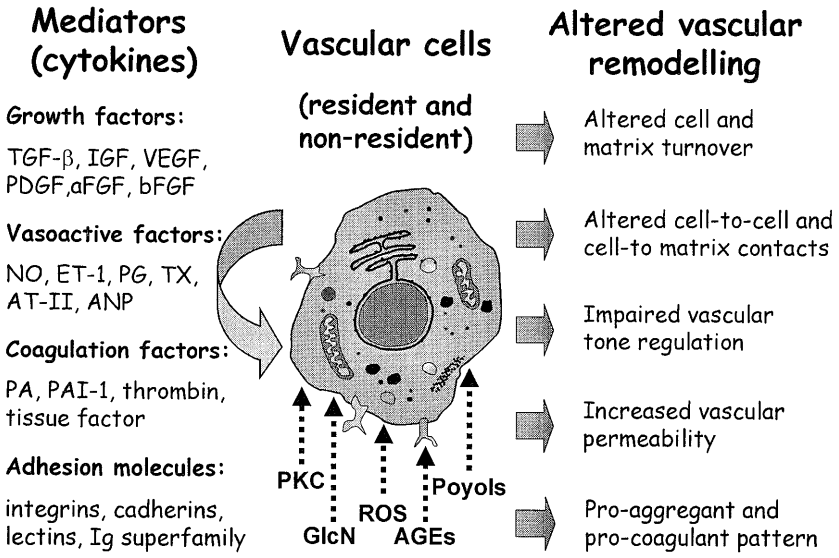

Fig. 4. Hyperglycaemia-dependent abnormal pattern of expression of mediators modulating altered vascular remodelling by acting in a paracrine and autocrine fashion at the level of resident and non-resident vascular cells. PKC, protein kinase C; GlcN, glucosamine; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; NO, nitric oxide; ET-1, endothelin-1; PG, prostaglandins; TX, thromboxane; AT-II, angiotensin II; ANP, atrial natriuretic peptide; PA, plasminogen activator; PAI-1, plasminogen activator inhibitor-1

An increased gene expression of tissue plasminogen activator (PA) and its inhibitor PAI-1, associated with reduced fibrinolytic potential, was shown in endothelial cells grown in high glucose [97]. Endothelial cells also exhibited an abnormal pattern of expression of adhesion molecules when exposed to high glucose concentrations, with an upregulation of E-selectin, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (VCAM-1) [98].

Hyperglycaemia-induced biochemical abnormalities and cytokine alterations. Numerous observations also indicate that high glucose-induced changes in the expression pattern of these cytokines are mediated by the biochemical abnormalities triggered by hyperglycaemia.

In cultured mesangial cells, AGEs were found to induce increased expression of growth factors such as TGF-β, IGF-I and platelet-derived growth factor (PDGF), associated with ECM overproduction and reduced cell proliferation [99–101]; these effects were prevented by co-incubation with anti-AGE-receptor antibodies [99, 101]. AGEs have also been shown to induce VEGF expression, in retinal pigment epithelial [102] and Muller [103] cells; production of TNF-α [104], IL-1 [104], PDGF [105], and IGF-I [106], in monocytes-macrophages; quenching of nitric oxide [107], activation of NF-κB [63], overexpression of VCAM-1 [108] and increased tissue factor and reduced thrombomodulin activity [109], in endothelial cells, and stimulation of p21(ras)-dependent MAPK and its downstream targets, NF-κB and the AP-1 complex, in vascular smooth muscle cells [110].

The inhibition of PKC has also been shown to reduce high glucose-induced TGF-β upregulation and ECM protein synthesis [111] and an ARI-prevented glucose-induced increase in TGF-β and PKC activity [112] in cultured mesangial cells.

The events following the AGE-RAGE interaction, including the activation of NF-κB and induction of VEGF synthesis, have been shown to be prevented by antioxidants [63,102]. Moreover, D-α-tocopherol treatment reduced high glucose-induced eicosanoid and fibronectin production in mesangial cells, together with increased DAG concentrations and PKC activity [113].

Glucosamine synthesis via GFAT was shown to mediate the high glucose-induced increased TGF-β1 production and consequent upregulation of fibronectin and proteoglycan synthesis in porcine mesangial cells [85] and SP1-dependent upregulation of TGF-β1 and PAI-1 expression in endothelial cells [84].

Dysregulation of vascular remodelling

This altered expression of factors regulating vascular function and structure profoundly impairs the physiologic turnover of the vessel wall, thus leading to an abnormal process of vascular remodelling which underlies the vascular complications of diabetes (Fig. 4).

Vascular remodelling under normal conditions. Under normal conditions [114], vascular tissue homeostasis

is maintained through a strictly regulated balance between cell and matrix compartments, which in turn is dependent upon an equilibrium between cell replication and death, on one side, and matrix synthesis and degradation, on the other.

Cell-to-cell and cell-to-matrix interactions also participate in this mechanism, because the adhesion molecules mediating these contacts are capable of influencing back the activity of the cells expressing them, the so called "outside-in signalling", as opposed to the "inside-out signalling", inherent in the classical adhesive function. While the resident vascular cells are in close contact with each other, their interaction with circulating elements, particularly leucocytes, is normally inhibited by shear stress, which opposes leukocyte adhesion together with a nonadhesive pattern of endothelial cell surface.

The vascular endothelium represents a barrier against the passage of macromolecules and cells from the lumen to the interstitium through the junctional and transcellular pathways of transendothelial transport. In addition, the basement membrane restrains permeation, which is dependent also on the Starling forces and the physico-chemical characteristics of circulating molecules.

The contractile elements of the vessel wall play a central role in regulating vascular tone and, hence, pressure and flow within the vessel lumen, which are regulated according to the perfusion pressure and tissue oxygen demands.

The balance between procoagulant and anticoagulant systems, i.e. platelet aggregation and intrinsic and extrinsic coagulation pathways, on one side, and natural anticoagulants and fibrinolytic pathways, on the other, operates to prevent thrombus formation.

Vascular remodelling in diabetes. In diabetes, these mechanisms are profoundly altered and hyperglycaemia seems to play a major role in this scenario, though other factors could be involved as well.

The balance between cell and matrix compartment is altered in vascular tissues of diabetic patients. Enhanced matrix deposition is a characteristic feature of diabetic vascular disease; indeed, increased basement membrane width is a widespread alteration occurring virtually in all vascular districts of the body. Abnormal matrix accumulation in the kidney is due to an imbalance between matrix synthesis, which is increased [115, 116], and degradation, that is impaired [117, 118], as shown also in the retina [119]. More complex are the changes involving the cell compartment, which can be either reduced, in absolute or relative terms, as a consequence of reduced proliferation and increased apoptosis, as shown in endothelial cells grown in high glucose [120, 121]. The increase in cellularity could result from either proliferation of resident cells, such as vascular smooth muscle cells, or inflow of non-resident cells, such as monocyte-macrophages.

Cell-to-cell and cell-to-matrix interactions are altered from the abnormal pattern of expression of molecules mediating these contacts and the imbalance between cell and matrix compartments, which is also influenced by the inflow of non-resident cells within the vessel wall. The increased integrin expression associated with upregulation of fibronectin production was shown to result in an increased attachment of endothelial cells to matrix proteins [122] and also to influence back cell proliferation through cytoskeletal changes [123]. An abnormal pattern of adhesion molecules on the endothelial cell surface is responsible for the increased leukocyte rolling and adhesion with subsequent migration within the vessel wall [98].

Loss of vascular barrier function is the result of several factors, including haemodynamic changes, endothelial dysfunction, basement membrane thickening and altered physico-chemical characteristics of plasma constituents [124]. Increased deposition of circulating macromolecules within the vessel wall is the consequence of this impairment of barrier function and represents another typical feature of diabetic vascular disease [125].

Vascular tone regulation is lost since the early phase of diabetes, with haemodynamic alterations characteristically changing throughout the natural history of the disease. Initially, blood flow is increased in several vascular districts, which applies particularly to the glomerular microvasculature [35, 37], due to arteriolar vasodilation strictly dependent upon blood glucose concentrations. This phenomenon has been attributed to several mechanisms, including polyol pathway [35–37] and PKC [75] activation, growth hormone (GH) and glucagon [126], ketone bodies [127], VEGF [128], nitric oxide [129], eicosanoids [130], etc. Later on, an impaired endothelium-dependent vasodilatation seems to prevail, due to the predominance of vasoconstrictors, such as ET-1, which are increased, over vasodilators, such as nitric oxide, which are reduced [131, 132].

A procoagulant pattern is the rule in diabetic vascular disease; it is the consequence of a complex series of changes involving all the mechanisms participating in the control of haemostasis [133, 134]. Spontaneous platelet aggregation was shown to be increased in patients with Type II diabetes, as compared with control subjects [135] and a large survey indicated that both PAI-1 and tissue PA are increased in blood of IGT or Type II diabetic patients compared with normal control subjects, and correlate positively with fasting insulin concentrations, thus supporting a role for insulin-resistance [136].

Vessel occlusion is the result of all these alterations, with increased vascular tone and vessel wall thickness, acellular capillaries and leukocyte obstruc-

tion prevailing in the microvasculature, and plaque formation with superimposed thromboembolic events predominating in the macrovasculature. The resulting ischaemia and hypoxia trigger an angiogenic response of vessels to compensate for the reduced flow downstream to the occlusion. However, the efficacy of this response is dependent on the type of vessel from which the new vessels form, i.e. the microvasculature or macrovasculature.

Target organ manifestations

Though these processes are widespread, they differ from tissue to tissue depending on the anatomical and functional characteristics of target organs, which determine the local pattern of expression of mediators and the prevailing alteration of tissue remodelling occurring in response to the same noxa, i.e. hyperglycaemia and the associated metabolic derangements.

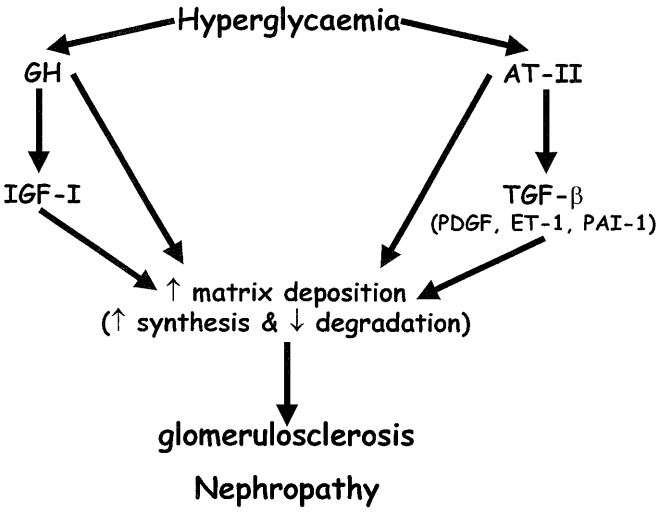

Fig. 5. Mechanisms of enhanced extracellular matrix accumulation in diabetic nephropathy. GH, growth hormone; AT-II, angiotensin II; PDGF, platelet-derived growth factor; ET-1, endothelin-1; PAI-1, plasminogen activator inhibitor-1

Nephropathy. In the kidney, the haemodynamic changes are an early feature, with the increase in renal blood flow and glomerular capillary pressure leading to increased GFR and proteinuria [137, 138]. In addition, the augmented blood flow is partly responsible for the glomerular hypertrophy that characterises this early phase [138, 139]. This glomerular hypertrophy has been considered as the forerunner of mesangial expansion and progressive loss of kidney function, although this sequence is not invariable in humans [7]. In this view, the growth factors thought to be involved in the development of early hypertrophy would participate also in the induction of subsequent glomerular lesions in susceptible individuals [140].

With increasing duration of diabetes, proteinuria and mesangial expansion gradually develop. Proteinuria is initially selective, with prevailing excretion of negatively charged proteins, such as albumin and the subclass 4 of IgG [141, 142]. This phenomenon seems to be related to a progressive loss of fixed anionic charges at the glomerular level, due to a reduced glomerular content or sulfation of glycosaminoglycans [143, 144].

Both the thickening of glomerular basement membrane and the expansion of the mesangial region result from an increased deposition of matrix, whereas the role of altered cell growth and turnover remains to be elucidated. A major contribution to matrix accumulation comes from the upregulation of its synthesis, as shown by the progressive increase in the gene expression of matrix components in glomeruli isolated from experimental diabetic rats, as compared with non-diabetic control animals [115, 116].

Two growth factor axes have been implicated in this alteration, namely the GH/IGF-I and the angiotensin II (AT-II)/TGF-β axes, the latter also leading to an upregulation of PDGF, ET-1 and PAI-1 (Fig. 5). All these cytokines take part in the increased matrix deposition and mesangial expansion, though a local upregulation of TGF-β, possibly triggered by the activation of the renin-angiotensin system, seems to play a major role.

Upregulation of TGF-β was shown to occur in glomeruli of patients with diabetic glomerulosclerosis [145] and also in animals with experimental diabetes, in both the early and advanced stages of glomerular disease [115, 116, 146, 147]. Likewise, an upregulation of this growth factor has been found in several human and experimental fibrotic disorders of the kidney and other organs [148]. Blocking of TGF-β action [149, 150] resulted in the prevention of experimental glomerulonephritis.

The relation between the prosclerotic cytokine TGF-β and the activation of the renin-angiotensin system has been demonstrated by a report on cells exposed to AT-II which showed increased mRNA expression for TGF-β and various matrix components [150]. Moreover, the upregulation of matrix production induced by AT-II was prevented by an antibody against TGF-β, thus proving that the effects of AT-II on matrix turnover are mediated through TGF-β [151]. When rats with experimental glomerulonephritis were treated with the ACE inhibitor enalapril and/or the AT1 receptor antagonist losartan, the increased matrix accumulation and TGF-β expression occurring in this model were considerably reduced [152]. This indicates that the beneficial effect of pharmacological AT-II blockade might not entirely depend on the blood pressure lowering and intrarenal haemodynamic effects but also on this inhibition of TGF-β-mediated matrix deposition. Moreover, that these abnormalities were not completely reversed

even by maximal doses of these agents [152] suggests that other factors are involved in the pathogenesis of mesangial matrix accumulation in diabetes.

At variance with TGF-β, the renal IGF system seems to be activated only in experimental diabetic and post-nephrectomy glomerulopathy [153, 154]. In particular, studies in spontaneous or streptozotocin-induced diabetic animals consistently showed that the kidney content of IGF-I increases during the first few days after diabetes induction and returns to normal concentrations thereafter [153, 154], thus suggesting that IGF-I is involved in the initial renal hypertrophy associated with diabetes. Most of these studies [155, 156] did not detect a parallel increase of IGF-I mRNA expression, thus raising the question of the source of IGF-I accumulating within the kidney. This can be either local, due to increased translation of IGF-I mRNA, or circulating, due to an increased sequestration of IGF-I by IGF receptors or binding proteins (IGFBPs). IGF receptors mRNA [156] and IGFBP expression [157] were found to be increased in short-term diabetes, though these data were not confirmed by other studies [155, 158]. At variance with the early stages of the disease, very few studies have investigated the renal IGF system in advanced diabetes. IGF-I gene expression was found to be unchanged both in glomeruli isolated from streptozotocin-induced diabetic rats with up to 24 weeks of disease duration [146] and in whole kidney samples obtained from genetically obese diabetic (*db*/*db*) mice with established nephropathy [159]. The importance of the GH/IGF-I axis is supported by the observation that mice, transgenic for a mutated GH (called bGH-G119 K), which functions as a GH antagonist, do not develop glomerular disease and do not show upregulation of TGF-β and collagen IV gene expression, at variance with the wild-type animals, when both groups were rendered diabetic [160]. Similar findings were obtained with a pegylated GH-receptor antagonist (G120K-PEG), which reduced albuminuria and led to a return to normal of glomerular volume in experimental diabetic rats [161].

Retinopathy. In the retina, haemodynamic changes also occur early in the course of the disease and are responsible for the initial lesions detected at fundus examination. Increased blood flow and vascular permeability are observed a few weeks after the induction of diabetes in rats [36, 37], though the increment in regional flow has been questioned. Later on, a progressive loss of endothelial cells and pericytes takes place and leads to the formation of acellular capillaries, responsible for areas of local ischaemia. A study has provided the first evidence of this phenomenon in human diabetes, in both retinal endothelial cells and pericytes [162].

The extension of ischaemia within the retinal tissue signs the progression from background to proliferative retinopathy. Hypoxia is a potent stimulus for the angiogenic cytokine VEGF, which is invariably increased at this stage, as shown in the ocular fluids of patients with active proliferative retinopathy [163]. The VEGF response, which is also directly stimulated by hyperglycaemia and associated biochemical abnormalities, such as AGE accumulation [102, 103] and PKC activation [164], is very active and results in excess angiogenesis (Fig. 6). IGF-I appears to play a permissive role in VEGF action at the level of MAPK activation [165]. The participation of these growth factors in the angiogenic response to retinal ischaemia was shown in a mouse model of neonatal retinal neovascularization. In this model, intravitreal injection of antisense oligonucleotide against murine VEGF was capable of preventing new vessel formation, as compared with noncomplementary antisense oligonucleotide [166]. The same effect on angiogenesis was produced by injection of soluble VEGF-receptor chimeric proteins [167] or treatment with an anti-IGF-I receptor-antibody, which blocked the permissive action of IGF-I on VEGF [165].

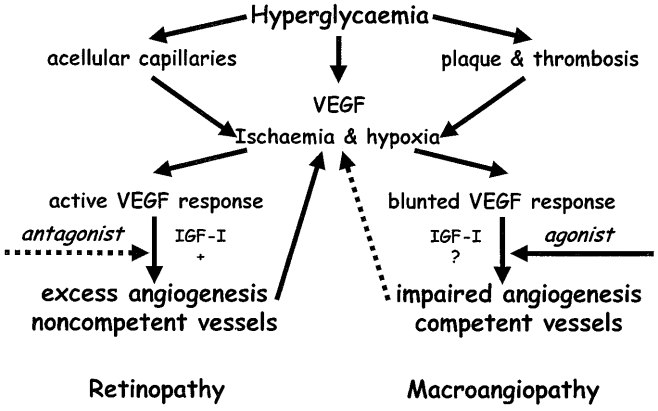

Fig. 6. Mechanisms and significance of angiogenesis in diabetic retinopathy and macroangiopathy. VEGF, vascular endothelial growth factor

Unfortunately, the new vessels formed at this level are not competent and very fragile, since they originate from retinal capillaries or venules. As a consequence, they are not capable of compensating for reduced blood flow and also break easily, thus leading to haemorrhage, which rapidly precipitates the impairment of vision.

Based on the conclusion that neovascularization exerts a detrimental effect on the course of retinopathy, several therapeutic approaches have been proposed for inhibiting angiogenesis at the retinal level [168] (Table 2). These agents could be delivered intravenously but because of the need for local action and the potentially harmful effect elsewhere at arterial level, it would be preferable to administer these intraocularly or intravitreally, though these procedures might cause adverse (local) effects. Preliminary re-

Table 2. Therapeutic approaches for angiogenesis inhibition in diabetic and other proliferative retinopathy

Therapeutic approaches	Delivery strategies
Inhibition of angiogenesis	Intravenous
– talidomide	– systemic
– angiostatin	– regional
– endostatin	*Local*
Blockade of angiogenic growth factors (VEGF)	– intraocular
– α-VEGF	– intravitreal
– VEGF oligonucleotides	
– VEGF receptor chimeric proteins	
– PKC inhibitors	
Inhibition of signal transduction	
– α-IGF-I receptor	
– octreotide	
– PKC inhibitors	
Inhibition of endothelial cell contacts	
– $\alpha_v\beta_3$ integrin blockers	
– MMP inhibitors	

MMP, matrix metalloproteinases

Table 3. Therapeutic approaches for angiogenesis stimulation in diabetic and other occlusive vascular diseases

Therapeutic approaches	Delivery strategies
→Angiogenic growth factors	*Intravenous*
– VEGF	– systemic
– aFGF	– regional
– bFGF	*Local*
– angiopoietin 1	– vascular wall (during balloon angioplasty)
given as:	– intra-myocardial (pericardial or endocardial route)
1. Recombinant protein	
2. Protein-encoding cDNA	
– naked (plasmid-contained)	
– viral vector-contained	

VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; aFGF, acidic fibroblast growth factor

sults in a small group of diabetic patients treated with octreotide, a somatostatin analogue, which can mimic the effect of hypophysectomy, indicate that retinal deterioration is slowed down, as compared with untreated subjects [169].

Macroangiopathy. In the arteries, the first alteration in the natural history of macroangiopathy seems to be the "endothelial dysfunction", a complex derangement of endothelial cell function linked to hyperglycaemia but also to other factors acting in the metabolic syndrome, such as dyslipidemia, hypertension and insulin resistance. All the functions of endothelial cells are involved in this alteration, including cell and matrix turnover, production of vasoactive agents and coagulation factors, expression of adhesion molecules and barrier properties, which shift from an antiatherogenic to a proatherogenic pattern [125, 170].

Increased lipid and lipoprotein concentrations and, particularly, oxidative and glycoxidative modification play a major part in plaque formation [171, 172]; modified LDL bind to the MSR instead of the LDL receptor, thus leading to the formation of foam cells [171, 172]. Once plaque is formed, it can remain quiescent indefinitely or progress more or less rapidly towards rupture and thrombus formation on its surface [173, 174]. The importance of changes in the expression of factors modulating thrombogenesis and fibrinolysis at plaque level is indicated by the clear antifibrinolytic pattern resulting from a decrease of PA and an increase of its inhibitor PAI-1 in specimens from coronary arteries [175]. Thrombosis and embolic dissemination are the main causes of large artery obstruction in diabetic and non-diabetic patients [176].

The response to ischaemia is similar to that observed at microvascular level in that it is mainly mediated by an upregulation of VEGF. At variance with retinopathy, however, the response of VEGF is blunted in peripheral and coronary arteries and neovascularization is impaired, despite that it leads to the formation of competent vessels, which are capable of compensating for the reduced flow downstream from the occlusion (Fig. 6). This has been shown in a model of hindlimb ischaemia in the NOD mouse, in which neovascularization could be improved by gene therapy with adeno-VEGF [177].

Therefore, the main therapeutic approach for stimulating angiogenesis [178] (Table 3) at the level of peripheral and coronary arteries is the use of angiogenic growth factors, the so called "therapeutic angiogenesis". These agents can be delivered intravenously or even better locally. This approach has been attempted in patients with diabetic and non-diabetic occlusive angiopathy of the peripheral and coronary circulation, with encouraging results [179, 180].

Conclusions

The question arises as to how all these devastating effects of diabetes at the vascular level can be prevented.

First of all, tight metabolic control remains the milestone intervention in vascular complications and new tools for achieving true euglycaemia should be actively searched for, because the more upstream the intervention the less are the consequences of long-term diabetes.

Efficient prevention and therapy of diabetic vascular complications also requires the search for and treatment of independent risk factors, which can affect vessels independently of hyperglycaemia. These factors include the known risk factors for cardiovascular disease, i.e. hypertension, dyslipidaemia and the other features of metabolic syndrome related to insulin resistance, cigarette smoking, sedentary life-

style, positive family history, etc. Moreover, the identification of markers of susceptibility to one or more of these sequelae would allow more aggressiveness in the achievement of tight glycaemic control and search for initial lesions in those patients who are predisposed to complications than in those who are not.

Because residual hyperglycaemia is the rule even with the most intensive hypoglycaemic intervention in the majority of patients, blockade of biochemical events triggered by intracellular disposal of excess glucose could be required. However, agents inhibiting the polyol pathway, PKC activity, AGE formation and oxidative stress have not been proven so far to be useful in preventing or treating vascular complications in humans. The elucidation of the interrelation and hierarchy among the various metabolic and biochemical abnormalities associated with hyperglycaemia might indicate the correct therapeutic approach at this level of the pathogenetic cascade.

Finally, due to the district specificity of pathogenetic mechanisms operating in diabetic vascular disease, the more downstream we plan the intervention the more targeted to an individual tissue it has to be. Therefore, the understanding of the relation between hyperglycaemia, the altered pattern of expression of various mediators and the dysregulated vascular remodelling at the level of the target tissues would allow design of therapeutic approaches targeted to the individual tissue and to the relevant biochemical, functional and structural alterations.

Acknowledgements. This lecture was given at the 36th Annual Meeting of the European Association for the Study of Diabetes (EASD), held in Jerusalem, Israel, September 17–21, 2000, by Professor U. Di Mario, who was awarded the Castelli Pedroli Prize. The studies cited in this article were supported by grants from the Ministry of University and Scientific and Technological Research of Italy, the Ministry of Health of Italy, the National Council of Research of Italy, the Italian Society of Diabetology, the Lions Italian Association for Diabetes, the Telethon Foundation, Rome, Italy (D.66), the International Center for the Study of Diabetes, Rome, Italy, and the Diabetes, Endocrinology and Metabolism Foundation, Rome, Italy. The Authors are grateful to M. Bontà for preparing the manuscript.

References

1. The Diabetes Control and Complications Trial Research Group (1993) The effect of intensive therapy of diabetes on the development and progression of long-term complications in insulin-dependent diabetes. *N Engl J Med* 329: 977–986
2. UK Prospective Diabetes Study Group (1998) Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352: 837–853
3. Toeller M, Buyken AE, Heitkamp G, Berg G, Scherbaum WA (1999) Prevalence of chronic complications, metabolic control and nutritional intake in type 1 diabetes: comparison between different European regions. EURODIAB Complications Study group. *Horm Metab Res* 31: 680–685
4. Laasko M (1999) Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes* 48: 937–942
5. Krolewski AS, Kosinski EJ, Warram JH et al. (1987) Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. *Am J Cardiol* 59: 750–755
6. Krolewski AS, Warram JH, Rand LI, Christlieb AR, Busick EJ, Kahn CR (1986) Risk of proliferative diabetic retinopathy in juvenile-onset type I diabetes: a 40-yr follow-up study. *Diabetes Care* 9: 443–452
7. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR (1985) The changing natural history of nephropathy in type I diabetes. *Am J Med* 78: 785–794
8. Krolewski AS, Warram JH, Rand LI, Kahn CR (1987): Epidemiologic approach to the etiology of type I diabetes mellitus and its complications. *N Engl J Med* 317: 1390–1398
9. Hanefeld M, Fisher S, Julius U et al. (1996) The DIS Group: Risk factors for myocardial infarction and death in newly detected NIDDM: Diabetes Intervention Study, 11-year follow-up. *Diabetologia* 39: 1577–1583
10. Haffner SM, Mykkänen L, Festa A, Burke JP, Stern MP (2000) Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sinsitive prediabetic subjects. Implications for preventing coronary heart disease during prediabetic state. *Circulation* 101: 975–980
11. Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A (1999) Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study. *Diabetes Care* 22: 920–924
12. Reaven GM (1995) Pathophysiology of insulin resistance in human disease. *Physiol Rev* 75: 473–486
13. Aljada A, Dandonas P (2000) Effect of insulin on human aortic nitric oxide synthase. *Metabolism* 49: 147–150
14. Calles-Escandon J, Mirza SA, Sobel BE, Schneider DJ (1998) Induction of hyperinsulinemia combined with hyperglycemia and hypertriglyceridemia increases plasminogen activator inhibitor 1 in blood in normal human subjects. *Diabetes* 47: 290–293
15. Seaquist ER, Goetz FC, Rich S, Barbosa J (1991) Familiar clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320: 1161–1165
16. Earle K, Walker J, Hill C, Viberti GC (1992) Familiar clustering of cardiovascular disease in patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 326: 673–677
17. Oates PJ, Mylari BL (1999) Aldose reductase inhibitors: therapeutic implications for diabetic complications. *Expert Opin Investig Drugs* 8: 2095–2119
18. Orisio S (1999) Gene polymorphism of the renin-angiotensin system and progression of diabetic nephropathy. *J Nephrol* 1: 9–17
19. Wakisaka M, He Q, Spiro MJ, Spiro RG (1995) Glucose entry into rat mesangial cells is mediated by both Na(+)-coupled and facilitative transporters. *Diabetologia* 38: 291–297
20. Heilig CW, Concepcion LA, Riser BL, Freytag SO, Zhu M, Cortes P (1995) Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. *J Clin Invest* 96: 1802–1814

21. Pugliese G, Tilton RG, Williamson JR (1991) Glucose-induced metabolic imbalances in the pathogenesis of diabetic vascular disease. *Diabetes Metab Rev* 7: 35–59
22. Williamson JR, Chang K, Frangos M et al. (1993) Hyperglycemic pseudo hypoxia and diabetic complications. *Diabetes* 42: 801–813
23. Schleicher ED, Weigert C (2000) Role of the hexosamine biosynthetic pathway in diabetic nephropathy. *Kidney Int* 58 [Suppl 77]: S13–S18
24. Koya D, King GL (1998) Protein kinase C activation and the development of diabetic complications. *Diabetes* 47: 859–866
25. Brownlee M (1996) Advanced protein glycosylation in diabetes and aging. *Ann Rev Med* 46: 223–234
26. Baynes JW (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes* 40: 405–412
27. Giugliano D, Ceriello A, Paolisso G (1996) Oxidative stress and diabetic vascular complications. *Diabetes Care* 19: 257–267
28. Engerman RL, Kern TS, Larson ME (1994) Nerve conduction and aldose reductase inhibition during 5 years of diabetes or galactosemia in dogs. *Diabetologia* 37: 141–144
29. Sima AAF, Bril V, Nathaniel V et al. (1988) Regeneration and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy treated with sorbinil. *N Engl J Med* 319: 548–555
30. Asahina T, Kashiwagi A, Nishio Y et al. (1995) Imapired activation of glucose oxidation and NADPH supply in human endothelial cells exposed to H_2O_2 in high-glucose medium. *Diabetes* 44: 520–526
31. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, King GL (1994) Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase C pathways in diabetes and hypergalactosemia. *Diabetes* 43: 1122–1129
32. Mauer SM, Steffes MW, Azar S, Brown DM (1989) Effects of sorbinil on glomerular structure and function in long-term diabetic rats. *Diabetes* 38: 839–846
33. Tilton RG, Pugliese G, LaRose LS et al. (1991) Discordant effects of the aldose reductase inhibitor, sorbinil, on vascular structure and function in chronically diabetic and galactosemic rats. *J Diabetes Complications* 5: 230–237
34. Engerman RL, Kern TS (1993) Aldose reductase inhibition fails to prevent retinopathy in diabetic and galactosemic dogs. *Diabetes* 42: 820–825
35. Bank N, Mower P, Aynedjian HS, Wilkes BM, Silverman S (1989) Sorbinil prevents glomerular hyperperfusion in diabetic rats. *Am J Physiol* 256: F1000–F1006
36. Pugliese G, Tilton RG, Speedy A et al. (1990) Vascular filtration function in galactose-fed versus diabetic rats: the role of polyol pathway activity. *Metabolism* 39: 690–697
37. Tilton RG, Chang K, Pugliese G et al. (1989) Prevention of hemodynamic and vascular (albumin) filtration changes in diabetic rats by aldose reductase inhibitors. *Diabetes* 38: 1258–1270
38. MacGregor LC, Matschinsky FM (1985) Treatment with aldose reductase inhibitor or with myo-inositol arrests deterioration of the electroretinogram of diabetic rats. *J Clin Invest* 76: 887–889
39. Nishikawa T, Edelstein D, Brownlee M (2000) The missing link: a single unifying mechanism for diabetic complications. *Kidney Int* 58 [Suppl 77]: S26–S30
40. Nishikawa T, Edelstein D, Du X-L et al. (2000) Normalizing mitochondrial superoxide production blocks three pathways of hyperglycemic damage. Nature 404: 787–790
41. Lal MA, Körner A, Matsuo Y et al. (2000) Combined antioxidant and COMT inhibitor treatment reverses renal abnormalities in diabetic rats. *Diabetes* 49: 1381–1389
42. Koya D, Haneda M, Kikkawa R, King GL (1998) d-alpha tocopherol treatment prevents glomerular dysfunctions in diabetic rats through inhibition of protein kinase C-diacylglycerol pathway. *Biofactors* 7: 69–76
43. Cameron NE, Cotter MA (1999) Effects of antioxidants on nerve and vascular dysfunction in experimental diabetes. *Diabetes Res Clin Pract* 45: 137–146
44. Hammes HP, Bartmann A, Engel L, Wülfroth P (1997) Antioxidant treatment of experimental diabetic retinopathy in rats with nicanartine. *Diabetologia* 40: 629–634
45. Bursell S-E, Clermont AC, Aiello LP (1999) High-dose vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes. *Diabetes Care* 22: 1245–1251
46. Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P, Creager MA (1996) Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 97: 22–28
47. Astley S, Langrish-Smith A, Southon S, Sampson M (1999) Vitamin E supplementation and oxidative damage to DNA and plasma LDL in type 1 diabetes. *Diabetes Care* 22: 1626–1631
48. Baynes JW, Thorpe JW (1999) Role of oxidative stress in diabetic complications. A New perspective from an old paradigm. *Diabetes* 48: 1–9
49. Koschinsky T, He CJ, Mitsuhashi T et al. (1997) Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci U S A* 94: 6474–6479
50. Vlassara H (1997) Recent progress in advanced glycation end products and diabetic complications. *Diabetes* 46 [Suppl 2]: S19–S25
51. Edelstein D, Brownlee M (1992) Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine. *Diabetes* 41: 26–29
52. Soulis-Liparota T, Cooper M, Papazoglou D, Clarke B, Jerums G (1991) Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozotocin-induced diabetic rat. *Diabetes* 40: 1328–1334
53. Vlassara H, Fuh H, Makita Z, Krungkrai S, Cerami A, Bucala R (1992) Advanced glycosylation products induce complex vascular dysfunction in normal animals: a model for diabetic and aging complications. *Proc Natl Acad Sci USA* 89: 12043–12047
54. Hammes H-P, Martin S, Federlin K, Geisen K, Brownlee M (1991) Aminoguanidine inhibits the development of experimental diabetic retinopathy. *Proc Natl Acad Sci USA* 88: 11555–11558
55. Cameron NE, Cotter MA, Dines K, Love A (1992) Effects of aminoguanidine on peripheral nerve function and polyol pathway metabolites in streptozotocin-diabetic rats. *Diabetologia* 35: 946–950
56. Singh R, Barden A, Mori T, Beilin L (2000) Advanced glycation end-products: a review. *Diabetologia* 44: 129–146
57. Wolfenbuttel BHR, Boulanger CM et al. (1992) Breakers of advanced glycation end products restore large artery properties in experimental diabetes. *Proc Natl Acad Sci USA* 95: 4630–4634
58. Neeper M, Schmidt AM, Brett J et al. (1992) Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 267: 14998–15004

59. Horiuchi S, Higashi T, Ikeda K et al. (1996) Advanced glycation end products and their recognition by macrophage and macrophage-derived cells. *Diabetes* 45 [Suppl]: S73–S76
60. Li YM, Mitsuhashi T, Wojciechowicz D et al. (1996) Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80 K-H membrane proteins. *Proc Natl Acad Sci USA* 93: 11047–11052
61. Vlassara H, Li YM, Imani Y et al. (1995) Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex. *Mol Med* 1: 634–646
62. Thornalley PJ (1998) Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. *Cell Mol Biol* 44: 1013–1023
63. Bierhaus A, Chevion S, Chevion M et al. (1997) Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. *Diabetes* 46: 1481–1490
64. Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM (1997) Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J Biol Chem* 272: 17810–17814
65. Park L, Raman KG, Lee KJ et al. (1998) Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med* 4: 1025–1031
66. Wautier JL, Zoukourian C, Chappey O et al. (1996) Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. *J Clin Invest* 97: 238–243
67. Lougheed M, Lum CM, Ling W, Suzuki H, Kodama T, Steinbrecher U (1997) High affinity saturable uptake of oxidized low density lipoprotein by macrophages from mice lacking the scavenger receptor class A type I/II. *J Biol Chem* 272: 12938–12944
68. Stitt AW, Burke GA, Chen F, McMullen CB, Vlassara H (2000) Advanced glycation end-product receptor interactions on microvascular cells occur within caveolin-rich membrane domains. *FASEB J* 14: 2390–2392
69. Pugliese G, Pricci F, Leto G et al. (2000) The diabetic *milieu* modulates the AGE-receptor complex in the mesangium by inducing or up-regulating galectin-3 expression. *Diabetes* 49: 1249–1257
70. Pricci F, Leto G, Amadio L et al. (2000) Role of galectin-3 as a receptor for advanced glycosylation endproducts. *Kidney Int* 58 [Suppl 77]: S31–S39
71. Cohen MP, Ziyadeh FN (1996) Role of Amadori-modified nonenzymatically glycated serum proteins in the pathogenesis of diabetic nephropathy. *J Am Soc Nephrol* 7: 183–190
72. Ziyadeh FN, Han DC, Cohen JA, Guo J, Cohen MP (1998) Glycated albumin stimulates fibronectin gene expression in glomerular mesangial cell: involvement of the transforming growth factor-beta system. *Kidney Int* 53: 631–638
73. Cohen MP, Sharma K, Jin Y et al. (1995) Prevention of diabetic nephropathy in db/db mice with glycated albumin antagonists. A novel treatment strategy. *J Clin Invest* 95: 2338–2345
74. Scivittaro V, Ganz MB, Weiss MF (2000) AGEs induce oxidative stress and activate protein kinase C-beta(II) in neonatal mesangial cells. *Am J Physiol* 278: F676-F683
75. Ishii H, Jirousek MR, Koya D et al. (1996) Amelioration of vascular dysfunctions in diabetic rats by an oral PKC β inhibitor. *Science* 272: 728–731
76. Koya D, Haneda M, Nagakawa H et al. (2000) Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC β inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *FASEB J* 14: 439–447
77. Nakamura J, Kato K, Hamada Y et al. (1999) A protein kinase C-β-selective inhibitor ameliorates neural dysfunction in streptozotocin-induced diabetic rats. *Diabetes* 48: 2090–2095
78. Kowluru RA, Jirousek MR, Stramm L, Farid N, Engerman RL, Kern TS (1998) Abnormalities of retinal metabolism in diabetes or experimental galactosemia: V. Relationship between protein kinase C and ATPases. *Diabetes* 47: 464–469
79. Inoguchi T, Battan R, Handler E, Sportsman JR, Heath W, King GL (1992) Preferential elevation of protein kinase C isoform βII and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. *Proc Natl Acad Sci USA* 89: 11059–11063
80. Koya D, Jirousek MR, Lin YW, Ishii H, Kuboki K, King GL (1997) Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest* 100: 115–126
81. Hempel A, Maasch C, Heintze U et al. (1997) High glucose concentrations increase endothelial cell permeability via activation of protein kinase Cα. *Circ Res* 81: 363–371
82. Hebert LJ, Daniels MC, Zhou J et al. (1996) Overexpression of glutamine-frucose-6-phosphate aminotransferase in transgenic mice leads to insulin resistance. *J Clin Invest* 98: 930–936
83. Kolm-Litty V, Tippmer S, Haring HU, Schleicher E (1998) Glucosamine induces translocation of protein kinase C isoenzymes in mesangial cells. *Exp Clin Endocrinol Diabetes* 106: 377–383
84. Du XL, Edelstein D, Rossetti L (2000) Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci USA* 97: 12222–12226
85. Kolm-Litty V, Sauer U, Nerlich A, Lehmann R, Schleicher ED (1998) High glucose-induced transforming growth factor-β1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. *J Clin Invest* 101: 160–169
86. Filippis A, Clark S, Proietto J (1997) Increased flux through the hexosamine biosynthesis pathway inhibits glucose transport acutely by activation of protein kinase C. *Biochem J* 324: 981–985
87. Haller H (1997) Postprandial glucose and vascular disease. *Diabet Med* 14 [Suppl 3]: S50–S56
88. Pugliese G, Pricci F, Romeo G et al. (1999) Autocrine and paracrine mechanisms in the early stages of diabetic nephropathy. *J Endocrinol Invest* 22: 708–735
89. Hoffman BB, Sharma K, Zhu Y, Ziyadeh FN (1998) Transcriptional activation of transforming growth factor-beta1 in mesangial cell culture by high glucose concentration. *Kidney Int* 54: 1107–1116
90. Pugliese G, Pricci F, Locuratolo N et al. (1996) Increased activity of the insulin-like growth factor system in mesangial cells cultured in high glucose conditions: relation to glucose-enhanced extracellular matrix production. *Diabetologia* 39: 775–784

91. Williams B, Gallacher B, Patel H, Orme C (1997) Glucose-induced protein kinase C activation regulates vascular permeability factor mRNA expression and peptide production by human vascular smooth muscle cells in vitro. *Diabetes* 46: 1497–1503
92. Cosentino F, Hishikawa K, Katusic ZS, Lüsher TF (1997) High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation* 96: 25–28
93. Chakravarthy U, Hayes RG, Stitt AW, McAuley E, Archer DB (1998) Constitutive nitric oxide synthase expression in retinal vascular endothelial cells is suppressed by high glucose and advanced glycation endproducts. *Diabetes* 47: 945–952
94. Park J-Y, Takahara N, Gabriele A et al. (2000) Induction of endothelin-1 expression by glucose. An effect of protein kinase C activation. *Diabetes* 49: 1239–1248
95. Pricci F, Pugliese G, Menè P et al. (1996) Regulatory role of eicosanoids in extracellular matrix overproduction induced by prolonged exposure to high glucose concentrations in cultured rat mesangial cells. *Diabetologia* 39: 1055–1062
96. Williams B, Schrier RW (1993) Glucose-induced protein kinase C activity regulates arachidonic acid release and prostaglandin production by cultured rat mesangial cells. *J Clin Invest* 92: 2889–2896
97. Maiello M, Boeri D, Podestà F et al. (1992) Increased expression of tissue plasminogen activatpor and its inhibitor and reduced fibrinolytic potential of human endothelial cells cultured in elevated glucose. *Diabetes* 41: 1009–1015
98. Morigi M, Angioletti S, Imberti B et al. (1998) Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. *J Clin Invest* 101: 1905–1915
99. Doi T, Vlassara H, Kirstein M, Yamada Y, Striker GE, Striker LJ (1992) Receptor-specific increase in extracellular matrix production in mouse mesangial cells by advanced glycosylation end products is mediated via platelet-derived growth factor. *Proc Natl Acad Sci USA* 89: 2873–2877
100. Crowley ST, Brownlee M, Edelstein D et al. (1991) Effects of nonenzymatic glycosylation of mesangial matrix on proliferation of mesangial cells. *Diabetes* 40: 540–547
101. Pugliese G, Pricci F, Romeo G et al. (1997) Up-regulation of mesangial growth factor and extracellular matrix synthesis by advanced glycation endproducts (AGEs) via a receptor-mediated mechanism. *Diabetes* 46: 1881–1887
102. Lu M, Kuroki M, Amano S et al. (1998) Advanced glycation end products increase retinal vascular endothelial growth factor expression. *J Clin Invest* 101: 1219–1224
103. Hirata C, Nakano K, Nakamura N et al. (1997) Advanced glycation end products induce expression of vascular endothelial growth factor by retinal Muller cells. *Biochem Biophys Res Commun* 236: 712–715
104. Vlassara H, Brownlee M, Manogue KR, Dinarello CA, Pasagian A (1988) Catechin/TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodeling. *Science* 240: 1546–1548
105. Kirstein M, Brett J, Radoff S, Ogawa S, Vlassara H (1990) Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: role in vascular disease of diabetes and aging. *Proc Natl Acad Sci USA* 87: 9010–9014
106. Kirstein M, Aston C, Hints R, Vlassara H (1992) Receptor-specific induction of insulin-like growth factor I in human monocytes by advanced glycosylation end product-modified proteins. *J Clin Invest* 90: 439–446
107. Bucala R, Tracey KJ, Cerami A (1991) Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. *J Clin Invest* 87: 432–438
108. Schmidt AM, Hori O, Chan JX et al. (1995) Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. *J Clin Invest* 96: 1395–1403
109. Esposito C, Gerlach H, Brett J, Stern D, Vlassara H (1989) Endothelial receptor-mediated binding of glucose-modified albumin is associated with increased monolayer permeability and modulation of cell surface coagulant properties. *J Exp Med* 170: 1387–1407
110. Satoh H, Togo M, Hara M et al. (1997) Advanced glycation endproducts stimulate mitogen-activated protein kinase and proliferation in rabbit vascular smooth muscle cells. *Biochem Biophys Res Commun* 239: 111–115
111. Studer RK, Negrete H, Craven PA, DeRubertis FR (1995) Protein kinase C signals thromboxane induced increases in fibronectin synthesis and TGF-beta bioactivity in mesangial cells. *Kidney Int* 48: 422–423
112. Ishii H, Tada H, Isogai S (1998) An aldose reductase inhibitor prevents glucose-induced increase in transforming growth factor-beta and protein kinase C activity in cultured mesangial cells. *Diabetologia* 41: 362–364
113. Tada H, Ishii H, Isogai S (1997) Protective effect of D-alpha tocopherol on the function of human mesangial cells exposed to high glucose concentrations. *Metabolism* 46: 779–784
114. Gibbons GH, Dzau VJ (1994) The emerging concept of vascular remodeling. *N Eng J Med* 330: 1431–1438
115. Fukui M, Nakamura T, Ebihara I, Shirato I, Tomino Y, Koide H (1992) ECM gene expression and its modulation by insulin in diabetic rats. *Diabetes* 41: 1520–1527
116. Pugliese G, Pricci F, Pesce CM (1997) Early, but not advanced, experimental diabetic glomerulopathy is reversed by pancreatic islet transplants. Correlation with glomerular extracellular matrix mRNA levels. *Diabetes* 46: 1198–1206
117. Reckelhoff JF, Tygart VL, Mitias MM, Walcott JL (1993) STZ-induced diabetes results in decreased activity of glomerular cathepsin and metalloproteinase in rats. *Diabetes* 42: 1425–1432
118. Nakamura T, Fukui M, Ebihara I, Osada S, Tomino Y, Koide H (1994) Abnormal gene expression of matrix metalloproteinases and their inhibitor in glomeruli from diabetic rats. *Ren Physiol Biochem* 17: 316–325
119. Roy S, Cagliero E, Lorenzi M (1996) Fibronectin overexpression in retinal microvessels of patients with diabetes. *Invest Ophthalmol Vis Sci* 37: 258–266
120. Lorenzi M, Cagliero E, Toledo S (1985) Glucose toxicity for human endothelial cells in culture: delayed replication, disturbed cell cycle, and accelerated death. *Diabetes* 34: 621–627
121. Baumgartner-Parzer SM, Wagner L, Pettermann M, Grillari J, Gessl A, Waldhausl W (1995) High-glucose-triggered apoptosis in cultured endothelial cells. *Diabetes* 44: 1323–1327
122. Roth T, Podestà F, Stepp MA, Boeri D, Lorenzi M (1993) Integrin overexpression induced by high glucose and by human diabetes: potential pathway to cell dysfunction in diabetic microangiopathy. *Proc Natl Acad Sci U S A* 90: 9640–9644
123. Podestà F, Roth T, Ferrara F, Cagliero E, Lorenzi M (1999) Cytoskeletal changes induced by excess extracellular matrix impair endothelial cell replication. *Diabetologia* 40: 879–886

124. Lum H, Malik AB (1994) Regulation of vascular endothelial barrier function. *Am J Physiol* 267: L223–L241
125. Laight DW, Carrier MJ, Anggard EE (1999) Endothelial cell dysfunction and the pathogenesis of diabetic macroangiopathy. *Diabetes Metab Res Rev* 15: 274–282
126. Hoogenberg K, Dullaart RP, Freling NJ, Meijer S, Sluiter WJ (1993) Contributory roles of circulatory glucagon and growth hormone to increased renal haemodynamics in type 1 (insulin-dependent) diabetes mellitus. *Scand J Clin Lab Invest* 53: 821–828
127. Trevisan R, Nosadini R, Fioretto P et al. (1987) Metabolic control of kidney hemodynamics in normal and insulin-dependent diabetic subjects. Effects of acetoacetic, lactic, and acetic acids. *Diabetes* 36: 1073–1081
128. Hovind P, Tarnow L, Oestergaard PB, Parving HH (2000) Elevated vascular endothelial growth factor in type 1 diabetic patients with diabetic nephropathy. *Kidney Int* 57 [Suppl 75]: S56–S61
129. Veelken R, Hilgers KF, Hartner A, Haas A, Bohmer KP, Sterzel RB (2000) Nitric oxide synthase isoforms and glomerular hyperfiltration in early diabetic nephropathy. *J Am Soc Nephrol* 11: 71–79
130. Craven PA, DeRubertis FR (1989) Role for local prostaglandin and thromboxane production in the regulation of glomerular filtration rate in the rat with streptozocin-induced diabetes. *J Lab Clin Med* 113: 674–681
131. Hopfner RL, Gopalakrishnan V (1999) Endothelin: emerging role in diabetic vascular complications. *Diabetologia* 42: 1383–1394
132. De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM (2000) Endothelial dysfunction in diabetes. *Br J Pharmacol* 130: 963–974
133. Matsuda T, Morishita E, Jokaji H et al. (1996) Mechanism on disorders of coagulation and fibrinolysis in diabetes. *Diabetes* 45 [Suppl 3]: S109–S110
134. Sobel BE (1999) Increased plasminogen activator inhibitor-1 and vasculopathy. A reconcilable paradox. *Circulation* 99: 2496–2498
135. Iwase E, Tawata M, Aida K et al. (1998) A cross-sectional evaluation of spontaneous platelet aggregation in relation to complications in patients with type II diabetes mellitus. *Metabolism* 47: 699–705
136. Meigs JB, Mittleman MA, Nathan DM et al. (2000) Hyperinsulinemia, hyperglycemia and impaired hemostasis. The Framingham Offspring Study. *JAMA* 283: 221–228
137. Mogensen CE (1972) Glomerular filtration rate and renal plasma flow in long-term juvenile diabetics without proteinuria. *B M J* 4: 257–259
138. Mogensen CE, Osterby R, Gundersen HJ (1979) Early functional and morphologic vascular renal consequences of the diabetic state. *Diabetologia* 17: 71–76
139. Seyer-Hansen K (1983) Renal hypertrophy in experimental diabetes mellitus. *Kidney Int* 23: 643–646
140. Flyvbjerg A (2000) Putative pathophysiological role of growth factors and cytokines in experimental diabetic kidney disease. *Diabetologia* 43: 1205–1223
141. Myers BD (1990) Pathophysiology of proteinuria in diabetic glomerular disease. *J Hypertens* 8 [Suppl]: S41–S46
142. Pietravalle P, Morano S, Cristina G et al. (1991) Charge selectivity of proteinuria in type I diabetes explored by Ig subclass clearance. *Diabetes* 40: 1685–1690
143. Parthasarathy N, Spiro RG (1982) Effect of diabetes on the glycosaminoglycan component of the human glomerular basement membrane. *Diabetes* 31: 738–741
144. Morano S, Guidobaldi L, Cipriani R et al. (1999) High glucose modifies heparansulphate synthesis by mouse glomerular epithelial cells. *Diabetes Metab Res Rev* 15: 13–20
145. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA (1993) Expression of transforming growth factor-β is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci USA* 90: 1814–1818
146. Nakamura T, Fukui M, Ebihara I et al. (1993) mRNA expression of growth factors in glomeruli from diabetic rats. *Diabetes* 42: 450–456
147. Park L-S, Kiyomoto H, Abboud SL, Abboud HE (1997) Expression of transforming growth factor-β and type IV collagen in early streptozotocin-induced diabetes. *Diabetes* 46: 473–480
148. Border WA, Noble NA (1994) Transforming growth factor-β in tissue fibrosis. *N Engl J Med* 331: 1286–1292
149. Border WA, Okuda S, Languino LR, Sporn MB, Ruoslahti E (1990) Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1. *Nature* 346: 371–374
150. Border WA, Noble NA, Yamamoto T et al. (1992) Natural inhibitor of transforming growth factor B protects against scarring in experimental kidney disease. *Nature* 360: 361–364
151. Kagami S, Border WA, Miller DE, Noble NA (1994) Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-β expression in rat glomerular mesangial cells. *J Clin Invest* 93: 2431–2437
152. Peters H, Border WA, Noble NA (1997) Targeting TGF-β overexpression in renal disease: maximizing the antifibrotic action of angiotensin II blockade. *Kidney Int* 54: 1570–1580
153. Flyvbjerg A, Thorlacius-Ussing O, Naeraa R, Ingerslev J, Ørskov H (1988) Kidney tissue somatomedin C and initial renal growth in diabetic and uninephrectomized rats. *Diabetologia* 31: 310–314
154. Bach LA, Jerums G (1990) Effect of puberty on initial kidney growth and rise in kidney IGF-I in diabetic rats. *Diabetes* 39: 557–562
155. Marshall SM, Flyvbjerg A, Frystyk J, Korsgaard L, Ørskov H (1991) Renal insulin-like growth factor I and growth hormone receptor binding in experimental diabetes and after unilateral nephrectomy in the rat. *Diabetologia* 34: 632–639
156. Werner H, Shen-Orr Z, Stannard B, Burguera B, Roberts CT Jr, LeRoith D (1990) Experimental diabetes increases insulin-like growth factor I and II receptor concentration and gene expression in kidney. *Diabetes* 39: 1490–1497
157. Flyvbjerg A, Kessler U, Dorka B, Funk B, Ørskov H, Kiess W (1992) Transient increase in renal insulin-like growth factor binding proteins during initial renal hypertrophy in experimental diabetes in rats. *Diabetologia* 35: 589–593
158. Ooi GT, Tseng LY-H, Tran MQ, Rechler M (1992) Insulin rapidly decreases insulin-like growth factor-binding protein-1 gene transcription in streptozotocin-diabetic rats. *Mol Endocrinol* 6: 2219–2228
159. Rabkin R, Fervenza FC (1996) Renal hypertrophy and kidney disease in diabetes. *Diabetes Metab Rev* 12: 217–241
160. Esposito C, Liu Z-H, Stricker GE et al. (1996) Inhibition of diabetic nephropathy by a GH antagonist: a molecular analysis. *Kidney Int* 50: 506–514
161. Flyvbjerg A, Bennet WF, Rasch R, Kopchick JJ, Scarlett JA (1999): Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomeru-

lar hypertrophy, and urinary albumin excretion in experimental diabetes in mice. *Diabetes* 48: 377–382
162. Mizutani M, Kern TS, Lorenzi M (1996) Accelerated death of retinal microvascular cells in human and experimental diabetic retinopathy. *J Clin Invest* 97: 2883–2890
163. Aiello LP, Avery LI, Arrigg PG et al. (1994) Vascular endothelial growth factor in ocular fluids of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 331: 1480–1487
164. Aiello LP, Bursell S-E, Clermont A et al. (1997) Vascular endothelial growth factor-induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an oral effective β-isoform-selective inhibitor. *Diabetes* 46: 1473–1480
165. Smith LEH, Shen W, Perruzzi C et al. (1999) Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. *Nat Med* 5: 1390–1395
166. Robinson GS, Pierce EA, Rook SL, Foley E, Webb R, Smith LEH (1996) Oligodeoxynucleotides inhibit retinal neovascularization in a murine model of proliferative retinopathy. *Proc Natl Acad Sci USA* 93: 4851–4856
167. Aiello LP, Pierce EA, Foley ED et al. (1995) Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proc Natl Acad Sci USA* 92: 10457–10461
168. Duh E, Aiello LP (1999) Vascular endothelial growth factor in diabetes. The agonist versus antagonist paradox. *Diabetes* 48: 1899–1906
169. Grant MB, Mames RN, Fitzgerald C et al. (2000) The efficacy of octreotide in the therapy of severe nonproliferative and early proliferative diabetic retinopathy. *Diabetes Care* 23: 504–509
170. Stehouwer CD, Lambert J, Donker AJ, van Hinsbergh VW (1997) Endothelial dysfunction and pathogenesis of diabetic angiopathy. *Cardiovasc Res* 34: 55–68
171. de Villiers WJ, Smart EJ (1999): Macrophage scavenger receptors and foam cell formation. *J Leukoc Biol* 66: 740–746
172. Yla-Herttuala S (1999) Oxidized LDL and atherogenesis. *Ann NY Acad Sci* 874: 134–137
173. Cooke JP (1997) The pathophysiology of peripheral arterial disease: rational targets for drug intervention. *Vasc Med* 2: 227–230
174. Campbell JH, Campbell GR (1994) Cell biology of atherosclerosis. *J Hypertens* 12 [Suppl]: S129–S132
175. Sobel BE, Woodcock-Mitchell J, Schneider DJ, Holt RE, Marutsuka K, Gold H (1998) Increased palsminogen activator inhibitor type 1 in coronary artery atherectomy specimens from type 2 diabetic compared with nondiabetic patients. *Circulation* 97: 2213–2221
176. Juhan-Vague I, Alessi MC, Vague P (1996) Thrombogenic and fibrinolytic factors and cardiovascular risk in non-insulin-dependent diabetes mellitus. *Ann Med* 28: 371–380
177. Rivard A, Silver M, Chen D et al. (1999) Rescue of diabetes-related impairment of angiogenesis by intramucular gene therapy with adeno-VEGF. *Am J Pathol* 154: 355–363
178. Kornowski R, Fuchs S, Leon MB, Epstein SE (2000) Delivery strategies to achieve therapeutic myocardial angiogenesis. *Circulation* 101: 454–458
179. Baumgartner I, Pieczek A, Manor O et al. (1998) Constitutive epression of phVEGF$_{165}$ after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation* 97: 1114–1123
180. Losordo DW, Vale PR, Symes JF et al. (1998) Gene therapy for myocardial angiogenesis. Initial clinical results with direct myocardial injection of phVEGF$_{165}$ as sole therapy for myocardial ischemia. *Circulation* 98: 2800–2804