Endothelial cell dysfunction in hyperglycemia: Phenotypic change, intracellular signaling modification, ultrastructural alteration, and potential clinical outcomes

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Hyperglycemia, the hallmark of Diabetes mellitus, is a major risk factor for endothelial dysfunction and vascular complication. In recent years, significant achievements have been made in understanding endothelial cell dysfunction triggered by high glucose concentration. The purpose of this review is to discuss the results of these recent developments. First, the remarkable plasticity of vascular endothelial cell in response to the high glucose insult is emphasized. This is evident through the switch in the cell’s normal quiescent profile into new phenotypes, endowed with biosynthetic, inflammatory, adhesive, proliferative, migratory, pro-atherogenic, and pro-coagulant properties, frequently overlapping each other. Then, we underline the imbalanced expression and activity of transcription and signaling pathways, and the intense metabolic activity that accompanies the change in endothelial cell phenotype. As an adaptation to the high glucose-induced biochemical modification, a severe alteration of cell structure is produced. The review concludes with the clinical outcomes of the subject, emphasizing the high glucose-associated endothelial cell dysfunctional molecules of potential for targeting, and for reducing the impact of hyperglycemia on vascular endothelium. Such interventions may lead to a more efficient therapy for the benefit of those diabetic patients who are at increased cardiovascular risk.

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1. Introduction

Endothelial cells (ECs) are flat epithelial cells that form a monolayer that lines the internal lumen of the blood vessels. In normal, physiological condition, ECs are exposed to circulating blood glucose levels in the range of ~3.6–5.8 mmol/L, which are tightly regulated as part of metabolic homeostasis. The cells are metabolically active, and produce mediators that affect vascular tone, cell adhesion, and the homeostasis of clotting, and fibrinolysis. Through its contribution to hemostasis, ECs ensure fluidity of the blood. In normal conditions, the phenotype of EC is characterized as quiescent, with turnover rates of the order of months to years. Latest reports have identified several molecules that control EC quiescence. These are the circulating form of human Bone Morphogenetic Protein-9 (BMP-9) [1], the cytosolic phospholipase A2-α when sequestered within Golgi apparatus [2], the transcription factor E2-2 (member of the basic helix–loop-helix family) [3], the very low-density lipoprotein receptor [4], and Angiopoietin 1 (Ang1), the ligand for the receptor tyrosine kinase Tie2 [5–7]. Shear stress is also a potent physiological regulator of EC quiescence. As a biomarker of vascular quiescence stands the anti-angiogenic R-ras gene expression [8].

2. High glucose concentration induces phenotypic switch and modifies the intracellular signaling in vascular endothelial cells

Exposure of vascular ECs to glucose levels over than 10 mmol/L (in vitro or in vivo, as in Diabetes mellitus) is regarded as a high glucose (HG) condition. The over normal glucose concentration perturbs cells homeostasis and biochemistry, triggering modifications both in large vessels (macrovascular) and in small blood vessels, such as arterioles, venules, and capillaries (microvasculature). As a consequence of HG concentration, EC quiescence is lost, cells acquire new phenotypes, their normal function is impaired, and “endothelial cell dysfunction” is installed. EC dysfunction is characterized by one or more of the following features: deficiency in bioavailable nitric oxide (NO), reduced endothelium-mediated vasorelaxation, hemodynamic deregulation, impaired fibrinolytic ability, enhanced turnover, overproduction of growth factors, increased expression of adhesion molecules and inflammatory genes, excessive generation of reactive oxygen species (ROS), increased oxidant stress, and enhanced permeability of the cell layer [9–12]. In examination of HG effects on vascular EC, one should also take into account that over physiological glucose concentrations lead to the accelerated formation of multiple biochemical
species unusual in physiological conditions. Among these are the nonenzymatic reactive Amadori products, 3-deoxyglucosone, diacylglycerol, methyglyoxal, advanced glycation end products (AGEs), ROS, and nitrosylated species, which further amplify the imbalance that portrays HG-associated EC dysfunction. ROS production also triggers the peroxidation of plasma membrane polyunsaturated fatty acids like linoleic acid and arachidonic acid, generating endogenous 4-hydroxy nonenal, a highly reactive carbonyl compound. The increased oxidative stress seems to be a common alteration, triggered by a Type 2 diabetes milieu, in which hyperglycemia is adjoined by insulin resistance, hyperinsulinemia, and dyslipidemia. There is a common agreement that endothelial dysfunction precedes the development of micro- and macrovascular complications associated with Type 2 diabetes, such as nephropathy, retinopathy, atherosclerosis, and coronary artery disease; the underlying mechanism includes the accelerated formation of AGEs, activation of protein kinase C, increased pro-inflammatory signaling, and impaired sensitivity of the PI 3-kinase signaling pathways [14]. Recent data show that endothiopathogenesis of EC dysfunction differs in Types 1 and 2 diabetes [15]; it is present at the earliest stages of metabolic syndrome and insulin resistance, and may precede the clinical diagnosis of Type 2 diabetes by several years [16].

A first issue examined in this overview is the remarkable plasticity of EC in HG conditions, allowing the transition of the normal quiescent profile into a spectrum of new biosynthetic, pro-inflammatory, pro-adhesive, pro-apoptotic, and/or senescent phenotypes; these frequently overlap, e.g. the biosynthetic phenotype is also adhesive, pro-inflammatory, and pro-atherogenic, while the pro-apoptotic phenotype is a senescent one. As a function of the duration of HG exposure (in vitro) and of circulating glucose level (in vivo) vascular ECs gradually turn into biosynthetic cells, endowed with an over developed rough endoplasmic reticulum (rER); however, in this condition, the protein folding process within rER might be affected, and the endoplasmic reticulum stress is installed [17]. In time, and also as a function of glucose concentration, ECs enlarged and thickened their basal lamina by mechanisms that involve complex biochemical changes [18]. To this modification contribute TGF-β and its receptor ALK1 [19], fibronectin over expression [20], AGEs [21], as well as AGEs cross-linking to collagen molecules; the latter products are less sensitive to degradation, and promote extracellular matrix accumulation [22].

HG concentration also induces pro-inflammatory and pro-adhesive phenotypic changes of vascular ECs [23,24]; in these circumstances, cell surfaces express adhesion molecules (intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial selectin), interleukin (IL)-1β expression becomes up-regulated [24], and secretion of VEGF, IL-8, IL-6, and TNF-α attains significantly increased levels [25]. Along with the typical cytokines (TNF-α and IL-1β), and chemokines (such as the monocyte chemoattractant protein-1), the latest reports emphasize that the pro-inflammatory phenotype of EC is associated with an increased expression of inflammatory genes (e.g. the Neuronatin gene) [26], with the presence of ROS and phosphorylation of ERK1/2, c-Jun NH2 – terminal kinase (JNK), NF-kb [23,27,28], and of NF-kB transcription factor inhibitor IkBα [29]. Interestingly, the effect of HG on this phenotypic change is dependent on the anatomic position of the vessel, as well as on the duration of diabetes. Thus, regions of arteries exposed to low shear stress are susceptible to inflammation, whereas regions exposed to high shear stress are protected; in the latter areas, the transcription factor NF-E2-related factor 2 inhibits p38 phosphorylation, and suppresses EC dysfunction [30]. Moreover, the duration of diabetes selectively up-regulates the inflammatory genes expression, i.e. in short-term diabetes, the mRNA transcripts for chemokine ligands CCL2 and CCL5 were up-regulated in the aortic ECs, while at later stages of diabetes these genes were up-regulated in both the aortic and venous ECs [31].

HG significantly enhanced the migration of ECs (within the retina) concomitant with the sustained activation of the downstream prosurvival and promigratory signaling pathways, including Src kinase, phosphatidylinositol 3-kinase/Akt1/endothelial NO synthase, and ERKs [32]. Recent reports demonstrate that EC migration is NO-induced in a process, which implies the inactivation of the transcription factor FOXO3a and subsequent down-regulation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) [33]. Much of the current literature deals with the migratory properties of ECs as manifest in the angiogenesis process, with endothelial progenitor cells (EPCs) migration during vascular repairs. Although these issues are beyond the subject of this review, an interesting recent report underlines that Type 2 Diabetes mellitus impairs EPC migration, in a process linked to the stimulation of CXC receptor-4 (CXCR4) and activation of PI3K/Akt/enOS signaling pathway [34].

Interestingly, although hyperglycemia is acknowledged to be an independent risk factor for developing diabetes-associated atherosclerosis, the pro-inflammatory environment in diabetes is the critical factor conditioning the early pro-atherosclerotic actions of HG [35]. Reportedly, the presence of hyperglycemia stimulates expression of thioredoxin-interacting protein (TXNIP) [36] with a possible role in the EC pro-atherosclerotic response to extracellular diabetic-like environment [37].

Another imbalance associated with the effect of HG concentration is the promotion of a pro-coagulant condition of endothelium (by production of pro-coagulant mediators such as plasminogen activator inhibitor-1, fibrinogen, and P-selectin) associated with a reduced fibrinolitic activity [15,38]. Reportedly, Type 2 diabetes patients may be especially vulnerable to prothrombotic events when hyperglycemia is concurrent with systemic inflammation [39]. Not only is the endothelial cell affected by HG, but also the circulating cells. Thus, a recent report emphasizes that a glucose-regulated protein (GRP78) is involved in platelet deposition during interaction with the vascular wall [40].

High glucose concentration may also trigger two opposed phenotypic changes of vascular EC: in some circumstances, EC turns into proliferative, while in others, into apoptotic. An important player in the induction of EC proliferation is nicotinamide phosphoribosyltransferase (Nampt); which enables cells to resist HG concentration, and to use excess glucose to support replicative longevity and angiogenic activity [41]. For the retinal ECs exposed to HG, it was demonstrated that proliferation was accelerated as the number of pericytes gradually decreased [42]. The altered hemodynamic forces generated by changes in blood flow, influence also EC proliferation [43]. Although there is a common agreement on HG concentration as an inducer for EC apoptosis [44,45], the circumstances that favor this process are diabetes duration (via selective up-regulated caspase-1mRNA) [31], the sequential activation of ROS, JNK, and caspase-3 [46,47], the down-regulation of connexins Cx43 [48], Cx37, and Cx40 [49], and the presence of auto-antibodies in patients with macular edema or progression to albuminuria [50]. The intracellular consequences of EC apoptotic changes consist in DNA fragmentation, and mitochondrial dysfunction, manifest by alteration of membrane potential, release of cytochrome-c, and mitochondrial fragmentation, and these changes play a potential pathogenic role in mediating the risk of Type 2 Diabetes mellitus. Thus, defective or insufficient mitochondrial function may lead to the chronic accumulation of lipid oxidative metabolites that can mediate insulin resistance and secretory dysfunction [48,51,52].

Moreover, in HG condition, a senescent phenotype of EC occurs [53]; reportedly, cell turnover and oxidative stress are engaged in
this process by mechanisms involving telomere shortening [54]. Other contributors to the senescent phenotype of EC are the decreased expression of NAD⁺-dependent deacetylase sirtuin1, and the activation of p53 acetylation [55].

Taken together, the multitude of new phenotypes acquired by EC under the insult of HG concentration reveals remarkable cell plasticity, linked to the alteration of specific molecules and intra-
cellular pathways. Comprehensive recent reports advanced deeper into EC biochemistry in HG conditions, unveiling the mechanisms that underlie its intense metabolic activity.

3. High glucose concentration intensifies the metabolic activity of vascular endothelial cells

The intensification of metabolic activity of ECs exposed to HG is the result of an imbalance between up-regulation of several transcription and signaling factors, and down-regulation of other intracellular molecules. Examples of activated transcription factors are the aryl hydrocarbon receptor transcription factor [56], p300 (a transcriptional co-activator with histone acetyl transferase activity) [57], and COUP-TFI (the chicken ovalbumin upstream promoter-transcription factor II) [58]. In human umbilical vein ECs, HG has time-dependent effects on COUP-TFI, i.e. the short-term (60–240 min) HG stimulation increases its expression, while long term stimulation (48 h) down-regulates its expression [58]. Other laboratories reported that HG treatment and diabetes produced a deregulated activation of ETS (E-twenty six), one of the largest families of transcription factors; this activation blocks the functional activity of progenitor cells and their commitment toward the EC lineage [59]. Another activated transcription factor is FOXO1; the mechanism is triggered by HG, acting through oxidative stress pathway. Subsequently, activated FOXO1 promotes inducible nitric oxide synthase (iNOS)-dependent NO-peroxynitrite generation, which leads in turn to LDL oxidation and eNOS dysfunction [60]. Moreover, in diabetic rats, the activation of transcription factor FOXO1 was linked to retinal microvascular cell loss [61].

In HG conditions, as well as in diabetes, activation of EC intra-
cellular signaling pathways occurs. The recent data emphasize the activation of JAK2/STAT3 pathway and of Vascular Endothelial Growth Factor (VEGF) [62], of NAD(P)H oxidase followed by ROS generation [63], and of protein kinase C [13]. Among the last family of enzymes, the activation of PKC-α, -β1/2, and PKC-δ isomers is linked to the development of diabetes pathologies affecting both large vessels (in atherosclerosis and cardiomyopathy) and small vessels (in retinopathy, nephropathy, and neuropathy) [64].

Another modification induced by HG is the augmented expres-
sion of lipid peroxidation products [65], methylglyoxal (an AGE precursor molecule) [66], angiotensin II receptor 1 [67], neurophinps 75 receptor [68], Transient Receptor Potential ion channel protein 1 [69], poly(adenosine diphosphate-ribose) polymerase, [70], and fibronectin [20]. The intracellular calcium [Ca²⁺] concentration is also enhanced by HG condition [71]. A further characteristic of EC dysfunction triggered by HG consists in the increased production of vasoconstrictor prostanoids (endoperoxides and prostacyclin) that activate Thromboxane A2 (TP) receptors on the underlying SMCs, contributing to ampliﬁed vascular wall contractility [72].

In contradistinction to the above-mentioned activating effects, HG concentration down-regulates a variety of EC molecules. Examples are the AGER1 (an AGE-receptor that counteracts Receptor for Advanced Glycation End products (RAGE)) [73], UCP2 [63], and eNOS. As shown by the latest reports, the mechanisms that may account for the decline in eNOS activity consist in enhanced ROS formation by NADPH oxidase and uncoupled eNOS [74], diminished Hsp-90–eNOS interaction, due to the reaction between heat shock protein-90 and the inhibitor κB kinase (IKK) [75], and inhibition of enzyme activity of dimethylarginine dimethylaminohydrolase, resulting in an accumulation of asymmetric dimethylarginine; the latter competes with the eNOS substrate, L-arginine, and inhibits NO formation [76]. Such a mechanism explains the other characteristic of EC dysfunction in hyperglycemia and Diabetes mellitus, i.e. the impeded endothelium-dependent relaxation of the vascular wall. The mitochondria are also vulnerable in HG-exposed ECs; dysregulation in fuel-sensing molecules, AMP-activated protein kinase (AMPK), and the histone/protein deacetylase SIRT1 predisposes to Type 2 diabetes and atherosclerotic cardiovascular disease [77]. The cellular fueling system (the tricarboxylic acid oxidation cycle and the fatty acid β-oxidation pathway) is a target for various noxious stimuli, some generated within mitochondria themselves, such as the ROS [78]. Overproduction of the latter by mitochondrial electron transport chain serves as a causal link between elevated glucose and three major pathways responsible for hyperglycemic damage, i.e. the activation of the hexosamine pathway, the increased formation of AGE, and the activation of PKC isofoms [17].

Collectively, the imbalanced biochemical pathways (described above) portray the dysfunctional condition of EC facing HG concentra-
tion either in vitro or in vivo, as in diabetic vasculature. As an adaptation to the modified biochemistry and dysfunction, a modi-
fication to cellular structure is produced. In time, the structural modifications aggravate damaging the EC and the vascular wall.

4. High glucose concentration modifies vascular endothelial cell ultrastructure

To evaluate HG-induced alterations of EC structure, the basic features of cells’ normal ultrastructure are briefly mentioned. EC plasmalemma expose differentiated macro- and micro-domains and membrane-associated receptors; the cells contain coated vesicles and caveolae (formerly known as plasmalemmal vesicles) endowed with specific receptors, and transendothelial channels [79]. The EC is quiescent in physiologic conditions (see Section 1), display rather rare organelles involved in biosynthetic activities (rER and Golgi apparatus) or in degradations (such as multivesicular bodies, a lysosomal like compartment, and lysosomes), and produce a unique, thin basal lamina. The ultrastructural alterations induced by HG in vascular ECs are documented both by in vitro (human aortic ECs cultured for 1–2 weeks in a medium supplemented with 25 mM β-glucose) and in vivo studies (the vasculature of mice and golden Syrian hamsters at 6 weeks and 6 months, respectively after streptozocin injection) [80,81]. The common morphologic feature is the gradual and significant enrichment of biosynthetic organelles. As examples, the Golgi complex is evident in the aortic and capillary ECs (Fig. 1a and b), and the abundance in rER is obvi-
ous in ECs of the athero-susceptible aortic arch (Fig. 1c), of retinal venules (Fig. 1d), and of the femoral artery (Fig. 1e). Reportedly, dia-
betes imparts diffuse endothelial perturbation in both arterial and venous endothelium [31]. Morphologic evidence for the cells’ active metabolism is also provided through the presence of multivesicular bodies on electron-micrographs. One to three copies of these organelles are evident per EC in capillaries of retinal inner layer of diabetic animals (Fig. 1f).

As a result of prolonged and direct contact with the hyperglycem-
ic milieu, mitochondrial fragmentation occurs [51], and the cell-
ular cytoskeleton is reorganized; the latter occupies almost the entire cytoplasm in myocardial capillary ECs (Fig. 1g); others have reported recently the reorganization of actin filaments in human ECs exposed to nonenzymatically glycated bovine serum albumin [12]. In addition, the intact microtubule and actin cytoskeleton is
required for heparanase secretion by ECs exposed to hyperglycemia [23].

It is generally recognized that diabetes is associated with the thickening of the capillary EC basal lamina; we show here that basal lamina turns into a folded structure (Fig. 1h), and generates reduplications with a multilayer appearance (Fig. 1i); this phenomenon was also observed at the interstitial capillaries of diabetic golden Syrian hamsters [82] and at the endoneurial capillaries of diabetic cats [83]. Functionally, the thickening of EC basal lamina impairs the amount and selectivity of transport of metabolic products and nutrients between circulation and the tissues [84]. Together, the above ultrastructural modifications are associated with the biosynthetic phenotype of vascular ECs.

On electron-micrographs, the proliferative phenotype of endothelium is assessed by the presence of two centriols (paired organelles involved in mitosis) in close proximity to each other (Fig. 2). Since the ultrastructural changes appear as secondary to the biochemical alterations and EC dysfunction, in order to alleviate them, it is essential to find the optimal moment for therapeutic intervention, when the molecular alterations are still reversible. Examples for uncovering such issues are illustrated by ongoing research.

5. Potential clinical outcomes: toward the attenuation of ECs disturbances induced by hyperglycemia

The main goal of translational medicine is to target the disturbed molecules/pathways in therapies aimed at attenuating HG-triggered EC dysfunction. The majority of such strategies intend to reduce the oxidative stress generated in hyperglycemia/diabetes by using a plethora of antioxidant molecules. Mitochondria-derived ROS generation can be inhibited by Pigment Epithelium-Derived Factor (PEDF) that also decreases lipid peroxidation, and downregulates VEGF; thus it appears that PEDF treatment may be beneficial in diabetic retinopathy [62,65]. Moreover, azaserine functions as antioxidant and protects against hyperglycemic endothelial damage [23]. ROS generation in EC can be regulated by overexpression of certain molecules, such as transcription factor NF-E2-Related Factor-2, AGER1, peroxisome proliferator-activated receptor-γ-co-activator 1-α, and AMP-activated protein kinase (AMPK) [73,85–87]. Activation of the latter suppresses 26S proteasome-mediated degradation of GTP-cyclohydrolase and up-regulates mitochondrial UCP2, resulting in the inhibition of superoxide anion production and prostacyclin synthase nitration; the final result is the prevention of the oxidative stress induced by hyperglycemia and the normalization of EC function [63,88]. In line with this, the mammalian homolog of the fish calcium regulatory hormone stanniocalcin-1 attenuates endothelial superoxide anions generation and activation of inflammatory pathways, and maintains tight junction proteins expression, preserving the EC monolayer seal [28].

As for the attenuation of inflammatory pathways activation, a potential therapeutic method that improves vascular barrier function consists in targeting key signaling molecules that mediate endothelial-junction-cytoskeleton dissociation [89]. A further approach is to inhibit the activity of certain molecules in specific
vascular beds. Thus, in brain microvascular ECs inhibition of glyco-
gen synthase kinase 3β reduced adhesion molecules expression, and decreased endothelial leukocyte adhesion under inflammatory conditions [90]; in diabetic retinopathy, inhibition of α4 integrin/CD49d signaling pathway attenuated the diabetes-induced up-regulation of NF-κB activation, reduced leukocyte adhesion to EC surface, and thus may hold promise for the clinical activity [91]. Another useful molecule is sphingosine-1-phosphate that inducts the expression of MAP kinase phosphatase-3, inhibiting the high-glucose-mediated ERK1/2 phosphorylation and exerting an anti-
flammatory effect on diabetic ECs [92].

The accelerated proliferation of retinal ECs and the decrease in pericytes number can be prevented through the addition of bioac-
tive TGF-β and by aldose reductase inhibition [42]. In addition, enhancing endothelial Namp act activity may be beneficial in scenari-
os requiring ECs-based vascular repair and regeneration during HG, such as diabetes-related vascular disease [41].

Inhibition of ECs migration can be achieved by activating Acti-
vin Receptor-Like Kinase 1 [93]. Moreover, enhanced production of thrombospondin (TSP2) is described as an inhibitor of EC migra-
tion and capillary morphogenesis during neovascularization [94].

The latest reports also indicate possible strategies for reducing ECs apoptosis and senescence. Reportedly, the HG-induced apopto-
sis is reduced by fenofibrate [44] and is inhibited by quercetin sul-
fate/glucuronide, the metabolite of quercetin in blood [47]. Moreover, enhanced IGF-1 signaling inhibits glucose-induced apoptosis in human umbilical vein ECs by reducing mitochondrial dysfunc-
tion, and maintaining the mitochondrial retention of cyto-
ochrome-c [95]. The prevention of HG-induced EC senescence is ex-
erted by the activation of Sirt1 (a NAD-dependent deacetylase) or dis-
ruption of p53 pathway [55]. L-arginine also exerts an anti-
senescent effect on human umbilical vein ECs exposed to 33 mmol/L glucose (via the PI3K/Akt pathway), and the authors claim it might be a therapeutic agent for diabetic vascular compli-
cations [53]. In addition, statins and peroxynitrite scavengers pos-
sess the ability to reduce senescence in laboratory models of disease (reviewed in Ref. [54]).

In summary, three systems may at present alleviate HG-
induced vascular EC dysfunctional profiles: exposure to inhibitors of certain molecules/pathways or down-regulation of specific mol-
ecules, activation or over-expression of particular molecules and intra-
cellular pathways, and correction of biochemical alterations (if still reversible) towards rehabilitation of the quiescent condi-
tion. Although translational medicine may potentially target the intracellular molecules/pathways identified thus far (as shown above), the avenue is far from reaching an end. Still not well under-
stood are the time-dependence and the vascular bed specificity of HG-induced EC biochemical changes, and substantiation of mech-
anisms which operate in continuous, fenestrated or discontinuous endothelia. Less clear still are the intracellular pathways which lead to basal lamina reduplication in HG conditions. In addition, despite the recent progress made in reducing the HG-associated deleterious effects on EC, the existence of a causal relationship be-
tween telomere dysfunction and endothelial senescence is yet to be demonstrated [54].

6. Concluding remarks

This literature reviewed outlines the most recent mechanisms underlining EC dysfunction, triggered by over-physiological glucose concentration. Modified biochemistry is linked to the phenotypic switch of EC that acquires new properties (biosynthetic, inflamma-
tory, adhesive, migratory, pro-atherogenic, pro-coagulant, prolifer-
ative, apoptotic or even senescent), rather than overlapping each other. Such changes are associated with the diabetic vasculopathy of large vessels and microvasculature. The results of the ongoing studies emphasize substantial progress in identifying EC-
disturbed molecules/pathways under the insult of HG; and these may hold therapeutic potential, and be of benefit to the diabetic patient.

Finally, it is important to point out that the reported effects of HG concentration are not the outcome of a unique insult, but of a multitude of deleterious molecules formed in HG conditions. These include the nonenzymatic glycation products, the AGE-prote-
ins, the oxidant free radical species, among other components of the diabetic environment. Moreover, under insulin resistant condi-
tions, as in Type 2 Diabetes mellitus, increased insulin concentra-
tions and/or impaired insulin-signaling pathways may also contribute to endothelial dysfunction [13]. Therefore, the effects of HG emphasized in the largest part of the literature reviewed here should be viewed within the more complex perspective of the diabetic milieu.

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