Determinants of Cardiovascular Risk in Diabetes Beyond Hyperglycemia

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Abstract — A link between diabetes and cardiovascular disease (CVD) has clearly been established. Indeed, patients with type 2 diabetes have an increased risk of developing CVD and a worst prognosis after suffering acute ischemic events and at the same time CVD is the main cause of death and disability among diabetic patients. Despite that clinical management of type 2 diabetic patients has traditionally been focused in lowering blood glucose levels, recent clinical trials have demonstrated that the use of intensive treatments to achieve near normal blood glucose levels is insufficient to reduce the incidence of major cardiovascular events in these subjects. In the last years, numerous studies have suggested that alterations beyond hyperglycemia and commonly found in diabetic patients, including insulin resistance, inflammation, cellular stress, and a low endogenous regenerative capacity may contribute to increase cardiovascular risk in type 2 diabetic patients. The purpose of the following review is to examine the mechanisms by which these alterations may enhance cardiovascular complications.

Keywords — Cardiovascular risk, diabetes, ER stress, inflammation, insulin resistance, oxidative stress.


I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by the presence of increased blood glucose levels whose prevalence has been dramatically increased in the last years as a consequence of lifestyle modifications, particularly the proliferation of fat-rich diets and the lack of physical exercise. Indeed, on 2011 366.3 million people (8.3%) suffered diabetes worldwide and if the current trend continues it is estimated that by the year 2030 its prevalence will increase to 551.8 million people (9.9%) 1.

The link between diabetes and cardiovascular disease (CVD) has clearly been established as diabetic patients have a significantly higher risk of suffering CVD and a worst prognosis after suffering an acute ischemic event compared to non-diabetics 2-5, while, at the same time, CVD is the main cause of death and disability among these patients 1. Throughout the years, numerous alterations have been described in platelets 6-12, endothelial cells 8,11,13-15, smooth muscle cells 2,3,13, and hemostasis 8,11,16,17 that can potentially contribute to the increased cardiovascular (CV) risk observed in these patients by either altering vascular wall structure and function, accelerating atherosclerosis development, and increasing thrombosis (Fig. 1). Moreover, another aspect that can increase CV risk in diabetic patients is the fact that they show a higher incidence of other CV risk factors such as obesity, hypercholesterolemia, hypertension, and sedentarism 18. Indeed, these patients are more prone to clustering CV risk factors and with a stronger negative impact compared to that observed in the non-diabetic population 19.

Hyperglycemia has previously been associated with CVD and with acute event occurrence 20-22. However, in recent years three major multicentric randomized clinical trials [i.e. Action to Control Cardiovascular Risk in Diabetes (ACCORD) 23, 24, Action in Diabetes and Vascular Disease (ADVANCE) 25, and the Veterans Affairs Diabetes Trial (VADT) 26, 27] have demonstrated that reducing blood glucose levels is insufficient to reduce major cardiovascular event occurrence in type 2 diabetes patients. The three trials recruited patients with type 2 diabetes, over 40 years old, and either a history of macrovascular complications or presence of additional CV risk factors (basal characteristics for each population are summarized in Table 1) in order to analyze if reducing blood glucose levels using an intensive treatment reduced the incidence of major macrovascular events. Therefore, patients were randomly assigned to receive a standard therapy to reduce blood glucose levels or an intensive treatment to achieve near-normal glycated hemoglobin (HbA1c) levels (6.0-6.5%). In all cases, both groups significantly reduced their HbA1c levels and, as expected, intensive treatment groups showed significantly lower HbA1c levels compared to those of the standard groups. However, no significant differences were observed between groups in the incidence of
major macrovascular events in any of the trials (Table 1) and no differences were observed in all-cause mortality in the ADVANCE and VADT trials. In contrast, the ACCORD trial was stopped prior to completion when a significant increase in all-cause mortality was observed in the intensive group (Table 1).

Altogether, the ACCORD, ADVANCE, and VADT trials included over 20,000 patients with type 2 diabetes and provided solid evidence to suggest that achieving near-normal blood glucose levels does not reduce cardiovascular event incidence. In fact, within the last years, numerous studies have suggested that alterations beyond hyperglycemia and commonly found in diabetic patients, including insulin resistance, inflammation, cellular stress, and a low endogenous regenerative capacity may contribute to increase CV risk in type 2 diabetic patients.

II. INSULIN RESISTANCE

Type 2 diabetes is a complex multigenic disease highly related with obesity, characterized by a defect in insulin secretion that gradually develops into hyperglycemia. In a first stage, insulin sensitive tissues (particularly muscle, liver, and adipose tissue) lose their capacity to adequately respond to insulin, giving rise to insulin resistance. As a consequence, pancreatic β-cells increase insulin secretion to preserve normal glucose tolerance. However, when fasting glucose levels exceed 140mg/dl, β-cells become incapable of maintaining the increased insulin secretion rate and thus insulin concentrations are gradually reduced until impaired glucose tolerance occurs. In this manner, when hyperinsulinemia cannot compensate insulin resistance, relative lack of insulin leads to diabetes.
TABLE 1

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>ACCORD(^{24})</th>
<th>ADVANCE(^{25})</th>
<th>VADT(^{27})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intensive</td>
<td>Standard</td>
<td>Intensive</td>
</tr>
<tr>
<td>N</td>
<td>5128</td>
<td>5123</td>
<td>5571</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>62±3</td>
<td>62±3</td>
<td>66±6</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>38.7</td>
<td>38.4</td>
<td>42.6</td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
<td>10</td>
<td>10</td>
<td>7.9±6.3</td>
</tr>
<tr>
<td>Previous cardiovascular event (%)</td>
<td>35.6</td>
<td>34.8</td>
<td>32.2</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>8.3±1.1</td>
<td>8.3±1.1</td>
<td>7.5±1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93.5±18.7</td>
<td>93.6±18.7</td>
<td>78.2±16.8</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>32.2±5.5</td>
<td>32.2±5.5</td>
<td>28±5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>107±14</td>
<td>107±14</td>
<td>99±13</td>
</tr>
<tr>
<td>Pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>136±17</td>
<td>136±17</td>
<td>145±22</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75±11</td>
<td>75±11</td>
<td>81±11</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>183±42</td>
<td>183±42</td>
<td>-</td>
</tr>
<tr>
<td>LDL</td>
<td>105±34</td>
<td>105±34</td>
<td>121±40</td>
</tr>
<tr>
<td>HDL</td>
<td>-</td>
<td>-</td>
<td>49±14</td>
</tr>
<tr>
<td>Men</td>
<td>47±13</td>
<td>47±12</td>
<td>-</td>
</tr>
<tr>
<td>Women</td>
<td>38±10</td>
<td>39±10</td>
<td>-</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>156</td>
<td>154</td>
<td>-</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>14.3</td>
<td>13.7</td>
<td>14.2</td>
</tr>
<tr>
<td>Former</td>
<td>44.4</td>
<td>44.0</td>
<td>-</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>6.4</td>
<td>7.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Major macrovascular events</td>
<td>352</td>
<td>371</td>
<td>557</td>
</tr>
<tr>
<td>(p=0.16)</td>
<td>(p=0.32)</td>
<td>(p=0.14)</td>
<td></td>
</tr>
<tr>
<td>Death from any cause</td>
<td>257</td>
<td>203</td>
<td>498</td>
</tr>
<tr>
<td>(p=0.04)</td>
<td>(p=0.28)</td>
<td>(p=0.62)</td>
<td></td>
</tr>
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</table>

However, numerous studies have shown that alterations in insulin’s secretion and action not only contribute to diabetes progression but also can directly increase CV risk by inducing both vascular (endothelial and smooth muscle cells) and circulating (platelets and the coagulation system) alterations.

Besides its metabolic actions, insulin also plays a relevant role in vascular function. The endothelium is key for the maintenance of vascular homeostasis acting as a selective barrier between blood and the vessel wall and synthesizing and releasing a number of paracrine and autocrine factors. Endothelial cells synthesize nitric oxide (NO) and prostacyclin (PGI2), two potent platelet inhibitors, release tissue plasminogen activator that promotes fibrinolysis and synthesize and release plasminogen activator inhibitor-1 (PAI-1; the main inhibitor of fibrinolysis), altogether preventing thrombus formation. However, insulin resistance alters endothelial cells’ synthetic and secretory capacity, causing what is known as endothelial dysfunction. Indeed, it has been reported that obese insulin-resistant subjects with normal glucose tolerance have the same degree of endothelial dysfunction as patients with overt type 2 diabetes. Insulin regulates NO and endothelin-1 (ET-1; vasoconstrictor) production in healthy subjects, insulin increases NO-mediated vasodilation; however, in insulin-resistant subjects, insulin signaling through the phosphoinositide-3-kinase (PI3K) pathway is impaired, reducing its capacity to activate the endothelial nitric oxide synthase (eNOS) and thus eventually reducing NO production. Conversely, signaling through the mitogen-activated protein kinase (MAPK) remains unaltered in these subjects, increasing ET-1 production and consequently inducing vascular smooth muscle cells vasoconstriction. Additionally, reduced expression of β2-adrenergic receptor (β2AR) has been shown to contribute to impaired glucose tolerance in pancreatic β-cells, contributing to the development of type 2 diabetes, and a decline in adrenergic response has also been shown to impair vasodilation.

Insulin resistance has also been associated with increased platelet reactivity as observed in diabetic patients. Under healthy conditions, insulin reduces platelet responsiveness to various agonists by reducing intracellular calcium concentration and increasing intracellular cyclic adenosine monophosphate and cyclic guanosine monophosphate concentrations, which act together to reduce platelet aggregation by increasing PGI2 and NO synthesis, respectively. However, in insulin-resistant subjects, insulin is incapable of exerting these actions, increasing intracellular calcium and reducing platelet sensitivity to PGI2 and NO. Similarly, a recent study showed that whereas insulin inhibits platelet tissue factor (TF; the main initiator of the extrinsic coagulation cascade that leads to thrombin generation) synthesis in healthy subjects, in diabetic subjects TF expression was increased by 1.6 fold. All these alterations render diabetic platelets...
hyperactive, lowering their threshold for activation, increasing their consumption, and consequently stimulating thrombopoiesis\textsuperscript{38, 40}. In this regard, we have recently reported that type 2 diabetic rats [(i.e., Zucker diabetic fatty rats (ZDF)] have an increased thrombopoiesis (reflected by increased platelet counts and an increased platelet size), caused by an accelerated platelet death and confirmed by an increased number of circulating reticulated platelets, the youngest platelets that contain mRNA residues and show an increased size and reactivity. Importantly, these alterations were associated with higher thrombotic risk as assessed by in vivo intravital microscopy in both wild-type ZDF and in lean-normoglycemic controls transplanted with ZDF bone marrow.\textsuperscript{11} Moreover, we have also described that obese insulin-resistant normoglycemic rats show increased platelet counts and an increased platelet size which, in turn, are associated with an increased thrombosis. Most importantly, thrombotic risk is directly correlated with the degree of insulin resistance suggesting that a reduction of peripheral insulin resistance may contribute to reduce the overall thrombotic risk\textsuperscript{41}.

Additionally, impaired insulin action in macrophages can also promote atherosclerosis progression increasing the expression of CD36 and consequently the uptake of modified lipoproteins and promoting the formation of a necrotic core enhancing macrophage apoptosis\textsuperscript{34}.

Besides inducing alterations at the cellular level, insulin resistance also contributes to the establishment of a hypercoagulable-hypofibrinolytic state. As such, diabetic patients show increased circulating TF levels, which are directly modulated by both hyperglycemia and hyperinsulinemia. Indeed, a simultaneous increase of insulin and glucose levels results in a greater increase in TF-procoagulant activity.\textsuperscript{42} Besides contributing to platelet adhesion, von Willebrand factor (vWF) is also a transporter for coagulation factor VIII (fVIII) and levels of both fVIII and vWF have been found to be associated with insulin levels\textsuperscript{43}. Moreover, PAI-1 levels have been consistently associated with insulin levels in healthy subjects with insulin resistance as well as in type 2 diabetic patients\textsuperscript{44, 47} and epidemiological studies have reported a significant association between fibrinogen and insulin levels\textsuperscript{47-50}.

### III. INFLAMMATION

During the last years it has become clear that inflammation is a key feature in the pathogenesis of type 2 diabetes\textsuperscript{51-54}. Indeed, solid experimental, epidemiological, and clinical evidence has linked inflammation, or the molecules and networks integral to inflammatory responses, to the development and complications of type 2 diabetes. Several cross-sectional studies have shown that, in comparison with non-diabetic subjects, newly diagnosed or established type 2 diabetic patients have increased circulating levels of acute phase reactants [C-reactive protein (CRP), serum amyloid A, α1-acid glycoprotein, sialic acid, fibrinogen and PAI-1]\textsuperscript{55-58}, and inflammatory cytokines [interleukin (IL) 1β, IL-6, and tumor necrosis factor alpha (TNF-α)]\textsuperscript{55, 56, 59-62}. IL-1β, IL-6, and TNF-α activate inflammatory pathways that terminate in the activation of either the Jun N-terminal kinase (JNK) or the inhibitor of kB kinase (IKK) which in turn alter signaling downstream of the insulin receptor leading to insulin resistance. IKK can impair insulin signaling by two mechanisms: a) by phosphorylating the insulin receptor substrate protein 1 (IRS1) on serine residues and consequently reducing tyrosine kinase-mediated insulin signaling; or b) by phosphorylating the inhibitor of nuclear factor κB (IKB) causing its degradation and releasing nuclear factor κB (NF-κB) whose translocation to the nucleus activates TNF-α and IL-6, further promoting the inflammatory response\textsuperscript{63, 64}. On the other hand, JNK activation can also contribute to the development of insulin resistance either by phosphorylating IRS1 serine residues and/or by stimulating the expression of inflammatory genes in association with transcription factor activator protein 1 (AP-1)\textsuperscript{65, 66}.

Recently, evidences indicating that cells and molecules involved in the adaptive immune response are involved in the inflammatory activity that links obesity with insulin resistance have led researchers to suggest that type 2 diabetes, as type 1 diabetes, has an autoimmune component. However, even if the presence of adaptive immunity has been demonstrated, its involvement in the pathogenesis of type 2 diabetes remains elusive for various reasons a) no conclusive data suggests that β-cell death in type 2 diabetes is due to autoimmunity, b) low-grade inflammation affects various tissues suggesting an innate immune response rather than an autoimmune response, and c) evolution of an innate immune response into an adaptive immune response has only been described in adipose tissue and not in pancreatic β-cells.

The proinflammatory state present in type 2 diabetic patients greatly contributes to their increased CV risk as inflammation is critically involved in all stages of atherosclerosis development\textsuperscript{67-69}. Moreover, various clinical studies have shown that low-grade elevation of circulating proinflammatory molecules is associated with the development of myocardial infarction, stroke, and peripheral vascular disease as well as with cardiovascular mortality\textsuperscript{70-72} and inflammation has been shown to contribute to cardiac insulin resistance through various mechanisms, contributing to the development of diabetic cardiomyopathy, a progressive deterioration of cardiac function which develops independently of other risk factors including coronary heart disease\textsuperscript{73}.

However, studies performed in experimental animal models have suggested that pharmacological treatment can reduce diabetic low-grade inflammation. Long-term treatment with metformin (an oral antidiabetic drug of the biguanide class) reduced phosphoactive forms of NF-κB and JNK, attenuated NF-κB gene expression, and increased the expression of anti-inflammatory genes in the liver of aging mice\textsuperscript{74}. Moreover, evidences have also suggested that peroxisome proliferator-associated receptor alpha (PPAR-α) may also be a
target for the treatment of diabetic inflammation, as an anti-inflammatory activity of PPAR-α, mediated by an inhibition of NF-κB, has been described in the retina of streptozotocin-induced diabetic rats. These results are in line with the previous observation that fibrates (PPAR-α agonists) induce an enhanced CV risk reduction in diabetic patients and reduce CRP levels.

IV. CELLULAR STRESS

In recent years, two forms of cellular stress, endoplasmic reticulum (ER) stress and oxidative stress, have been shown to play an important role in diabetes progression as well as to increase systemic inflammation, altogether contributing to increase CV risk in these patients.

ENDOPLASMIC RETICULUM STRESS

The ER exerts numerous functions that are key for cell survival being responsible for folding and assembly of secreted and membrane proteins, participating in lipids and sterols synthesis, and being the main intracellular storage of free calcium. Protein folding is essential for proper protein function as misfolded proteins can maintain non-native interactions that can interfere with their structure and function. Indeed, the ER has developed a quality control system that guarantees that only properly folded proteins exit the ER to other intracellular organelles or to the cell surface, while misfolded proteins are either retained in the ER lumen forming complexes with molecular chaperones or sent to the proteasome to be degraded in a process called ER-associated degradation.

Protein folding is a dynamic process that is continuously adjusted through the integration of various signals and its efficiency depends on environmental, genetic, and metabolic conditions. Therefore, physiological states that increase protein demand or disturb protein folding cause an imbalance between the load of unfolded proteins that enter the ER to be processed and the capacity of the ER to process them, causing the accumulation of unfolded or misfolded proteins in the ER lumen, leading to ER stress, and triggering the unfolded protein response (UPR). The UPR is a dynamic network of signaling events activated to reduce the load of misfolded or unfolded proteins through various mechanisms in order to promote cell survival. However, when ER stress is persistent or excessive and the UPR is insufficient to restore homeostasis, the UPR can induce apoptosis either through induction of the CCAAT/enhancer-binding protein homologous protein (CHOP), inducing calcium release, or activating the apoptosis signal-regulating kinase.

ER stress activates three transmembrane signal transducers located in the ER that initiate the different branches of the UPR: the protein kinase RNA-like endoplasmic reticulum kinase (PERK), the inositol-requiring kinase 1 (IRE1), and the activating transcription factor 6 (ATF6). Under normal conditions, the intraluminal domains of these sensors are bound to the 78 KDa glucose related protein (GRP78) keeping them in an inactive state. However, as misfolded or unfolded proteins accumulate in the ER lumen, these sequester GRP78 releasing PERK, IRE1, and ATF6. The PERK branch of the UPR reduces global mRNA translation to prevent the influx of newly synthesized proteins to the stressed ER lumen. On the other hand, under stress conditions IRE1 cleaves X-box binding protein (XBP1) mRNA turning it into a high activity transcription factor that translocates to the nucleus inducing the expression of UPR target genes.

Previous studies have shown that chronic activation of the UPR can lead to cellular dysfunction and contribute to the development of both type 2 diabetes and atherosclerosis, suggesting an important role of ER stress in the increased CV risk observed in type 2 diabetes.
available for protein folding is reduced and the PERK branch of the UPR is activated to attenuate translation. On the contrary, when glucose levels rise UPR pathways are inhibited to accelerate translation and to allow proinsulin entry to the ER. However, in type 2 diabetes, chronic hyperglycemia increases insulin demand to compensate insulin resistance, overwhelming the ER secretion capacity, activating the UPR, and ultimately causing β-cell dysfunction further contributing to diabetes progression.

Regarding atherosclerosis, increasing evidence suggests that ER stress plays an important role promoting plaque progression and increasing plaque instability by adversely affecting both macrophages and vascular cells. Macrophage apoptosis increases plaque vulnerability by promoting necrotic core formation and chronic activation of the UPR has been shown to be one of the causes of increased macrophage apoptosis observed in advanced atherosclerotic lesions. Indeed, studies have shown that CHOP deficiency reduces macrophage death in advanced lesions and plaque necrosis in ApoE mice and a close correlation between CHOP expression and plaque vulnerability has been shown in human coronary artery lesions. On the other hand, exposure to oxidized phospholipids activates the UPR in endothelial cells and increased expression of IRE1, ATF6, and XBP1 has been observed in endothelial cells in athero-prone regions of disturbed blood flow. In smooth muscle cells, ER stressors have been shown to upregulate CHOP in vitro and treatment of fat-fed ApoE mice with the ER stress-inducing drug bortezomib reduced collagen and smooth muscle cell content and markedly increase necrotic cores in atherosclerotic plaques. Finally, we have recently described that alterations in platelet expression of ER stress proteins GRP78 and protein disulphide isomerase can also contribute to increase thrombosis by increasing the amount of active TF.

OXIDATIVE STRESS

Oxygen’s elevated reactivity allows it to participate in high-energy electron transfers supporting adenosine-5-triphosphate generation and at the same time renders it liable to attack any biological molecule. Therefore, the organism has developed a complex antioxidant defense system to protect against constant oxidative attacks. In this manner, oxidative stress can be defined as an alteration of the oxidant-antioxidant balance caused by an increased production of reactive oxygen species (ROS) or by deficiencies in antioxidant defenses.

ROS are a family of molecules with a high reactivity that include free radicals and non-radical intermediates such as superoxide (O$_2^-$), hydroxyl (HO$^-$), hydrogen peroxide (H$_2$O$_2$), NO, peroxyl radical (R-COO$^-$), peroxynitrite (ONOO$^-$) and hypochlorous acid (HOCl). When in homeostatic concentrations, ROS play important roles in cell function as they activate redox-sensitive transcription factors (i.e. AP-1, p53, and NF-κB) that regulate the expression of proinflammatory cytokines, cell differentiation, and apoptosis and activate protein kinases that promote cell survival and proliferation (i.e. ERK1/2 and apoptosis (i.e. p38MAPK and SAPK-JNK). One of the main sources of ROS is the mitochondrial respiratory chain. Along the chain, electron transfer between enzymes is not totally efficient causing electron leakage (mainly from complexes I and III) to molecular oxygen and consequently O$_2^-$ formation. Thus, the rate of O$_2^-$ formation depends on the number of electrons present and consequently increases under increased glucose and oxygen conditions. However, mitochondrial O$_2^-$ production is also increased in hypoxia as reduced oxygen availability (final electron acceptor for complex IV) causes electron accumulation. Protein folding in the ER is another important source of ROS, as disulphide bond formation is an oxidative process that can give rise to approximately 25% of O$_2^-$, even though this amount can be increased in cells with a high secretory activity and also under ER stress conditions. Other sources of ROS are enzymatic reactions involving nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, cytochrome P450, and other oxido-reductases. Conversely, the antioxidant defense system is composed by both enzymatic and non-enzymatic elements. Superoxide dismutases transform O$_2^-$ in H$_2$O$_2$, less reactive and capable of diffusing through cell and organelle membranes that acts as a second messenger, and H$_2$O$_2$ is then transformed into water by the action of catalase and glutathione peroxidase. Non-enzymatic defense elements include vitamins C and E, thioredoxin, ceruloplasmin, and transferrin.

As a consequence of chronic hyperglycemia, β-cells experience a process called glucotoxicity through which they show a gradual decrease of glucose-induced insulin secretion and insulin gene expression and eventually impaired cell function and death. This process is, at least partially, mediated by the accumulation of ROS as β-cells are particularly vulnerable to oxidative stress as they do not express catalase and show only low levels of glutathione peroxidase. Therefore, increased levels of ROS reduce proinsulin synthesis by decreasing mRNA expression through the inactivation of two β-cell-specific transcription factors (PDX1 and MafA) that regulate the expression of proinsulin genes and downstream genes required for β-cell differentiation, proliferation, and survival.

Besides contributing to diabetes progression, oxidative stress has also been shown to contribute to atherosclerotic-related thrombotic complications by increasing endothelial dysfunction and/or promoting platelet activation/aggregation. In endothelial cells, O$_2^-$ produced in the mitochondrial respiratory chain activates protein kinase C (PKC), which in turn activates membrane-associated NADPH-dependent oxidases further increasing O$_2^-$ production. Moreover, ONOO$^-$ (produced by the interaction between NO and O$_2^-$) oxidizes tetrahydrobiopterin, a co-factor for eNOS, uncoupling the enzyme and consequently reducing NO production and producing O$_2^-$ and asymmetric dimethylarginine, an endogenous inhibitor of eNOS. ONOO$^-$ also reduces...
endothelial production of PGI2, a vasodilating prostanoid that inhibits platelet aggregation. Similar to endothelial cells, ROS also activate platelet PKC, increasing O2 producing and reducing platelet nitric oxide synthesis.

Importantly, recent evidence suggests that ER stress, oxidative stress, and inflammation are interconnected through various mechanisms (Fig. 2) and that reducing either one will affect the others. This crosstalk has extensively been described in cells with a high secretory capacity, such as hepatocytes, macrophages, adipocytes, pancreatic β-cells, and oligodendrocytes, as these cells are particularly sensitive to ER stress or alterations in the metabolic status as they require trafficking of large amounts of proteins through the ER. Intracellular calcium, ROS, and NO have been demonstrated to be key mediators for the integration of these processes.

As previously stated, intermolecular disulphide bond formation is a process that requires oxidizing conditions and where O2 is the terminal electron acceptor of the electron transfer chain, thus leading to ROS formation. Consequently, an increase in the ER protein-folding load can lead to the accumulation of ROS triggering an inflammatory response activating transcription factors such as NF-κB, AP-1, and p53. On the other hand, accumulation of misfolded proteins in the ER lumen can cause calcium leakage from the ER. Released calcium then concentrates in the mitochondrial matrix causing depolarization of the inner mitochondrial membrane, disrupting electron transfer, and therefore increasing ROS production. These mitochondrial ROS can further increase calcium release from the ER, causing protein misfolding, and thus increasing ER stress.

NF-κB is a transcription factor that plays a key role in the onset of inflammation, whose activation has been linked to ER stress even though the mechanisms have not been completely elucidated. However, it has been proposed that activation of the UPR can contribute to NF-κB activation through various mechanisms. The PERK branch of the UPR attenuates translation and consequently, as the half-life of the IκB is much shorter than that of NF-κB, increases the NF-κB/IκB ratio releasing NF-κB to translocate to the nucleus. IRE1-mediated mechanisms have also been proposed for NF-κB activation by ER stress. IRE1 autophosphorylation induces a conformational change in its cytosolic domain that allows it to bind the TNF receptor-associated factor 2 (TRAF2). This IRE1-TRAF2 complex can recruit IKK which phosphorylates IκB inducing its degradation, thus increasing NF-κB translocation. Moreover, IRE1-TRAF2 can also recruit JNK kinase, which in turn induces the expression of inflammatory genes phosphorylating the AP-1 transcription factor.

V. REDUCED ENDOGENOUS REGENERATIVE POTENTIAL

Various studies have shown that endothelial progenitor cells (EPCs) derived from the bone marrow have the potential to regenerate the damaged endothelium and to home in ischemic tissues. EPCs reside in the bone marrow in a niche characterized by a low oxygen pressure and increased levels of stromal-derived factor 1 (SDF-1). As a consequence of vascular damage and/or hypoxia, damaged tissues secrete growth factors such as the hypoxia-inducible factor 1, the vascular endothelial growth factor, erythropoietin, and SDF-1 increasing their concentration above that encountered within the bone marrow. These factors in turn activate eNOS leading to NO production and allowing the translocation of EPCs into the peripheral blood. Once in the circulation, EPCs follow cytokine gradients towards damaged tissues where, once adhered, they can contribute to regeneration by: a) differentiating into mature cells to directly replace damaged tissue; b) differentiating into mature endothelial cells and participating in neo-vessel formation; or c) promoting neo-vessel formation in a paracrine manner by secreting cytokines and growth factors that promote angiogenesis.

Diabetes has been proven to seriously affect these endogenous repair mechanisms by altering EPCs number, mobilization, and functionality. Several studies performed in experimental animal models as well as in humans have demonstrated that diabetes reduces the number of EPCs and alters their functionality. Indeed, it has been shown that hyperglycemia reduces EPCs proliferation and increases EPCs apoptosis and that EPCs derived from diabetic patients have a lower in vitro angiogenic capacity. Moreover, mobilization of EPCs has also been shown to be reduced in diabetic patients and the previously described reduction of PI3K/Akt/eNOS pathway signaling caused by insulin resistance and the consequent reduction of NO production has been proposed as one of the underlying mechanisms. In fact, a recent meta-analysis has demonstrated that diabetes reduces EPCs mobilization in response to granulocyte colony stimulating factor. Altogether, these alterations of EPCs-mediated endogenous repair mechanisms could contribute to increase CV complications in diabetic patients.

VI. FUTURE PERSPECTIVES

The dramatic increase in the prevalence of diabetes observed during the last years has increased the need to improve clinical management of these patients in order to reduce their incidence of major cardiovascular events. In this context, failure of intensive glycemic control therapies to reduce diabetic macrovascular complications has pointed out that more attention should be paid to alterations beyond hyperglycemia. Given the evidence provided so far, a reduction of endoplasmic reticulum stress, oxidative stress, and/or inflammation through pharmacological or lifestyle interventions seems promising as, given the extensive crosstalk between these three alterations, reducing one can potentially reduce the others. However, extensive research is further required to determine the feasibility as well as the effectiveness of these therapeutic approaches.
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