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Pathogenesis and pathophysiology of accelerated atherosclerosis in the diabetic heart

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Abstract It has been established that atherosclerotic coronary artery disease is more frequent and more severe in diabetic compared to non-diabetic subjects, but the reason for the excess risk of developing coronary macroangiopathy in diabetes remains incompletely characterized. Various biochemical mechanisms speculated to being at the "heart" of diabetic cardiac and coronary macroangiopathy are reviewed in the present article. In doing so, this article presents evidence that the labyrinthine interactions of hyperglycemia, insulin resistance, and dyslipidemia in diabetes result in a pro-atherogenic phenotype. Furthermore, the diabetic milieu yields a complex (dys)metabolic environment characterized by chronic inflammation, procoagulability, impaired fibrinolysis, neovascularization abnormalities, and microvascular defects that cumulatively alter blood rheology, artery structure, and homeostasis of the endothelium. The contributory influences of these factors in the pathophysiology of coronary artery disease in diabetes are also discussed.

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Introduction

Diabetes mellitus (DM) is a disease of epidemiological impact, affecting over 200 million people worldwide and among the five leading causes of death in most developed countries [1]. With the link between DM and cardiovascular disease (CVD) being well established and morbidity and mortality rates reaching epic proportions, the disease represents one of the greatest medical and socio-economic challenges for this century.

From a cardiac perspective, heart disease in DM manifests as a result of unfavorable modulation at different levels causing vascular (angiopathy), myocardial (diabetic cardiomyopathy), and intracardial nervous (autonomic neuropathy) dysfunction. Coronary artery disease (CAD), which causes occlusion of the arteries that supply the heart, has been reported to be directly responsible for much of the affliction of CVD in patients with DM [2] and while it has been established that atherosclerotic CAD is more frequent and more severe in diabetic compared to non-diabetic subjects, the reason for the excess risk of developing coronary macroangiopathy in DM remains elusive.

It must be acknowledged that with a disease as multifaceted as diabetes, it remains a challenge to entangle the importance of different contributory insults. This significantly impedes enhancement of treatment and prevention options to reduce the considerable disability, premature mortality, loss of productivity, and burden on health care facilities associated with the disease. Since detailed understanding of the effects of DM on the vasculature is a

pre-requisite to the development of more effective therapeutic interventions and new clinical strategies for treatment of the disease, it is fitting to review the current state of knowledge, and understanding of the mechanisms through which diabetes promotes the progression of atherosclerosis and CAD. To this purpose, a bifid approach has been adopted wherein the first part of this two-tiered review is presented with evidence that the metabolic derangements in DM both independently and in synergy, contribute toward the pathogenesis of atherosclerosis. In the second part, the pathophysiology of coronary atherosclerosis in DM with regard to mechanisms involved in accelerated atherothrombosis is discussed.

Clinical presentation of diabetes mellitus

Diabetes mellitus is a progressive, debilitating disease characterized by hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or both [3]. On the basis of etiology, natural history, and clinical presentation of the disorder, the disease is classified into type 1 and type 2 DM. The more prevalent form is type 2 DM, previously known as "Non-Insulin-Dependent Diabetes Mellitus," accounting for 90–95% of diabetic individuals [4]. The underlying cause of type 2 DM is an impairment of insulin-mediated glucose uptake, i.e., insulin resistance (IR) or a defective secretion of insulin by pancreatic β cells. As age advances insulin secretion tends to decline. In this respect, it is noteworthy that most diagnoses of type 2 DM are made after the age of 40 years although the age of onset maybe several years earlier [5]. Factors initiating IR are multifactorial, cumulative, and are usually accelerated by genetic pre-disposition. For example, IR may be brought on by obesity and sedentary lifestyle acting on a substrate of genetic susceptibility [6]. IR clusters with a multitude of cardiovascular risk factors that collectively form a syndrome known as the IR/metabolic syndrome (MS). Despite differences in specific criteria and definitions there is consensus in the published literature regarding the major characteristics of MS, such as dyslipidemia, hypertension, and IR. The latter commonly precedes onset of overt diabetes [7, 8].

Type 1 DM, also known as "Insulin-Dependent Diabetes Mellitus" results from cellular-mediated autoimmune destruction of pancreatic islet β cells resulting in loss of insulin production [9]. Type 1 DM, previously known as juvenile-onset DM ranks as one of the most common childhood diseases in developed nations. The causative factors of type 1 DM continue to be under some speculation and a genetic vulnerability has been suggested [10].

The burden of cardiovascular diseases in diabetes mellitus

DM is a heterogeneous disorder that initiates a diverse spectrum of ill effects on the vasculature. Vasculopathy in DM has been divided into microvascular (ophthalmologic, renal, and neurologic) and atherothrombotic macrovascular complications (coronary, cerebrovascular, and peripheral vascular) that represent the main etiology for mortality and a large percent of morbidity in patients with DM. CVD refers to dysfunction of the heart and/or the blood vessels and includes stroke, peripheral vascular disease, and coronary artery disease [11].

In 2007, in the UK alone, it was reported that over 2.2 million people were affected by DM and an estimated 750,000 people went undiagnosed [12]. The incidence of type 2 DM is reaching alarming proportions with 100,000 new cases every year and by extrapolation, one person every 5 min [13]. CVD accounts for up to 80% of the premature mortality associated with DM and in the UK, diabetes is second only to smoking as the leading cause of CVD [14]. DM has been established as a major independent risk factor for CVD in current risk assessment algorithms, such as the Framingham risk score, the New Zealand risk calculator and the British Guidelines [15]. Diabetic patients frequently show signs of accelerated atherosclerosis, undergo acute coronary syndromes, myocardial ischemia (MI) with peripheral artery disease (PAD), and stroke [16]. As reported recently by Peter and colleagues [17], compared with a non-diabetic individual, a person with type 2 DM has a 2- to 4-fold increased risk of dying from MI or stroke and a 10- to 15-fold increased risk of a lower extremity amputation. Diabetic patients also have an adverse course following MI, with high rates of post-infarct failure and death [18]. Epidemiological studies have provided clear evidence of the negative influence of diabetes on the prevalence, severity, and prognosis of cardiovascular disease presenting as coronary heart disease (CHD), PAD, cardiac neuropathy, stroke, and cardiac heart failure. DM is also noted to result in cardiac dysfunction and heart failure with reduced and preserved systolic function independent of the severity of coronary artery lesions [19, 20]. These are the outcomes of a distinct disease process known as diabetic cardiomyopathy, which is a major complication of DM.

With the current explosion in diabetes prevalence, healthcare costs for treatment of cardiovascular complications alone have reached astronomical proportions. In the UK, 5% of the National Health Service (NHS) budget is currently spent on treating diabetes and its cardiovascular complications [12]. People with diabetes account for 10% of all hospital admissions (up to 20% in some age groups), 1.75 million bed days every year in the main medical and surgical specialties and 30% of coronary care admissions,

costing the NHS system an estimated £3.5 billion a year and a projected £9.6 million a day. These figures are expected to increase by more than 25% in the next 20–30 years [12].

Coronary artery disease in DM

There exists an unfortunate consensus in the literature over the association of diabetes, systemic atherosclerosis, and an increasing severity of large-vessel CAD. Despite advances in therapeutic strategies, compared to non-diabetic patients those with DM have a 2- to 4-fold increase in CAD and a greater extent of coronary ischemia [21]. In a study involving 2,253 patients undergoing diagnostic coronary angiography, type 2 diabetic patients displayed more severe and diffuse coronary atherosclerosis with a higher prevalence of three-vessel disease [22]. Other large scale trials, such as the "Thrombolysis and Angioplasty in Myocardial Infarction" (TAMI) and "Thrombolysis in Myocardial Infarction Phase II" (TIMI-II) showed a greater incidence of multi-vessel disease in the hearts of diabetics than in non-diabetic patients [23]. Furthermore, as indicated by a recent study, patients with type 1 diabetes also bear a disproportionate burden of CAD at all ages compared to the general population, exhibiting higher mortality rates from ischemic heart disease [24].

Atherosclerosis being the primary pathophysiological process underlying CAD, diabetics have a greater degree of atherosclerosis than non-diabetic patients and this has been confirmed by various angiographic and autopsy studies. Results of a population based autopsy study, conducted in 2002, showed occurrence of coronary atherosclerosis in 49% of diabetic decedents as opposed to 33% of non-diabetic decedents [25]. The study also found ventricular dilation, high grade atherosclerosis, and multi-vessel disease in the diabetic group along with an overall high prevalence of subclinical atherosclerosis without a history of CAD [25]. Similarly, Dortimer et al. [26] demonstrated that diabetic subjects had more triple vessel disease (43% vs. 25%), more significantly affected vessels (68% vs. 46%) and fewer normal vessels (11% vs. 27%). It has been reported that the diabetic heart develops atherosclerotic disease in a different distribution compared with non-diabetic subjects being more diffuse and peripherally extended [27]. Other comparative assessments report more severe coronary disease, more extensive coronary calcifications, a higher prevalence of left main stem disease, and reduced coronary collateral artery recruitment in diabetic patients compared to their non-diabetic counterparts [28].

Women with diabetes have an eight times greater risk of developing CAD and the overall mortality from heart disease is twice as great in men and is 4–5 times higher in women with than without DM [29]. From the Framingham study, it was deduced the relative risk for CAD in diabetic men and women 45–71 years of age is 2.1 and 5.1 times greater, respectively, compared to age-matched controls [30]. Following the Framingham heart study, many groups have developed mathematical models to assess the incidence of CAD in DM. Particularly noteworthy are the findings of a recent study that employed a set of equations to predict the probability of developing CAD in a subset of diabetic American Indians after accounting for all potential risk factors (including age, body mass index, low density lipoprotein (LDL) cholesterol level, current smoking status, ECG-evident left ventricular hypertrophy, etc.). It was deduced that DM was associated with a 2-fold higher CAD incidence in men and a 3-fold higher incidence in women compared with those without diabetes [31]. In light of the foregoing, the usual female advantage in risk from death by ischemic events, and CHD appears to be nullified in the context of diabetes [32].

In addition, diabetes appears to have an adverse impact on prognosis post-acute coronary events with diabetics accounting for over 20% of percutaneous coronary interventions and outcomes after intervention remaining significantly worse [33]. In the "Insulin Risk and Atherosclerosis Study," it was found that diabetic patients with no history of CAD are at equal risk for future myocardial ischemic events as non-diabetic patients in whom a clinical diagnosis of CAD has been made [34]. A similar trend was observed in another epidemiological study, wherein at 70 months after MI, survival rates were about 50% for men and 40% for women with diabetes, compared with 70% for men and 75% for women without diabetes [35]. A recent study that examined the effect of DM on CAD prognosis following insertion of drug eluting stents showed that coronary re-stenosis was more likely to occur in diabetic compared to non-diabetic patients [36]. Additionally, one-quarter of patients presenting with acute MI are diabetic and DM is also a predictor of ischemic stroke and heart failure, increasing overall cardiovascular risk in patients with heart failure [37]. These discoveries have led American and European consensus opinions to recommend DM as a "coronary risk equivalent" rather than a risk factor [24, 38].

Metabolic abnormalities underlying pathogenesis of coronary atherosclerosis in diabetes mellitus

It is recognized that cardiovascular dysfunction as a consequence of DM arises from dysregulated metabolism, preceding emergence of histopathological changes, and clinically detectable events [39]. Hyperglycemia and insulin resistance are the characteristic metabolic anomalies associated with DM [40] that render the arteries susceptible to atherosclerosis and CAD. Much research in the area has led to the identification of putative cellular and

subcellular mechanisms that contribute to the pathogenesis of atherogenesis in the heart. These mechanisms will be briefly outlined along with a discussion of dyslipidemia that is particularly relevant to type 2 DM.

Hyperglycemia

DM is associated with an increased propensity to systemic atherosclerosis and increasing severity of CAD with increasing hyperglycemia, as measured by glycosylated hemoglobin ($HBA1_c$) levels [15]. The results of clinical studies, such as the Epidemiology of Diabetes Interventions and Complications (EDIC) and Multiple Risk Factor Intervention Trial (MR-FIT), demonstrated that the major cardiovascular complications of DM are attributable to the excessively high levels of plasma glucose and that hyperglycemia is a risk factor in its own right [23, 41]. Results of the EDIC study also suggested that intensive blood glucose control of type 1 and type 2 diabetic patients is associated with a reduced degree of coronary atherosclerosis. Hyperglycemia is a hallmark of DM and it induces a variety of maladaptations at the cellular level of vascular tissue, which may in part account for macrovascular complications. In fact, chronic hyperglycemia has been indicated as the key factor in the pathogenesis of diabetic complications [42].

Four major mechanisms may be held accountable for pathological alterations in the diabetic vasculature as an effect of hyperglycemia including increased glucose flux through the polyol–sorbitol pathway, hexosamine pathway, formation of advanced glycation end products (AGE), and activation of protein kinase C (PKC).

The polyol–sorbitol pathway

In the polyol pathway, aldose reductase (AR) and sorbitol dehydrogenase catalyze the metabolism of glucose to sorbitol, and sorbitol to fructose, respectively. These reactions are accompanied by the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to $NADP^+$ and reduction of nicotinamide adenine dinucleotide (NAD^+) to hydrogenated NAD (NADH). That is,

$$\text{Glucose} \xrightarrow[\text{Aldose reductase}]{\text{NADPH}\rightarrow\text{NADP}^+} \text{Sorbitol} \xrightarrow[\text{Sorbitol dehydrogenase}]{\text{NAD}^+\rightarrow\text{NADH}} \text{Fructose}$$

Excess glucose flux through this pathway leads to the amplification of osmotic stress due to increased sorbitol accumulation and increased oxidative stress due to the depletion of NADPH and increase in the cytosolic NADH/NAD^+ ratio. The ensuing tissue hypoxia is termed "hyperglycemic pseudohypoxia." Sorbitol and fructose levels have been reported to be approximately 9-fold higher and NADH/NAD^+ levels are approximately 4-fold higher in diabetic hearts [43]. The exact relationship between the polyol pathway flux and atherosclerosis is only partially understood with studies suggesting a pathological role based on glucose levels. An important mechanism whereby increased polyol pathway flux promotes vascular damage is by increasing oxidative stress through a decrease in the production of the antioxidant glutathione. In the reduction of glucose to sorbitol, phosphorylated NADH (NAD(P)H, a cofactor of glutathione production) is consumed. In vivo experiments confirm this finding and a study on low density lipoprotein (LDL) receptor deficient diabetic mice showed increased atherosclerotic lesion size when overexpression of human AR was induced [44]. Other studies suggest an up-regulation of AR during foam cell formation and resultant increase in oxidative stress in the macrophages [45]. Experimental data show that inhibitors of AR can prevent the development of long-term diabetic complications in transgenic mice that overexpressed AR [46]. However, to the present day no clinical evidence suggests alleviation of atherosclerosis in diabetic patients by AR inhibition and the pathophysiological role of the polyol pathway in diabetic atherosclerosis has not been fully delineated [44].

The hexosamine pathway

In the hexosamine pathway, fructose-6-phosphate is converted to glucosamine-6-phosphate and finally to UDP (uridine diphosphate) *N*-acetyl glucosamine through the action of the enzyme L-glutamine:D-fructose-6-phosphate amidotransferase (GFAT). Shunting of excess glucose into this pathway has been shown to result in disruptions in transcription activity and normal enzyme function. Schematically, this effect can be described as follows:

$$\text{Fructose-6-phosphate} \xrightarrow{\text{GFAT}} \text{Glucosamine-6-phosphate} + \text{UDP-}N\text{-acetyl glucosamine}$$
$$\downarrow$$
$$O\text{-linked protein glycosylation}$$
$$\downarrow$$
$$\text{Altered enzyme function}$$

In bovine aortic endothelial cells, increased flux through the hexosamine pathway was shown to increase *N*-acetyl glucosamine. In tandem with *O*-linked glycosylation of transcription factor Sp-1, this was found to increase levels of transcription of pro-inflammatory cytokines, such as transforming growth factor-β (TGF-β) and plasminogen activator inhibitor-1 (PAI-1) [47]. Also, it has been shown that hyperglycemia-induced inhibition of nitric oxide synthase (NOS) activity can be reversed by inhibition of the enzyme GFAT in streptozotocin (STZ)-induced diabetic rats [48]. NOS is responsible for the synthesis of the prominent mediator of vascular tone, nitric oxide (NO). Inhibition of GFAT by oligonucleotides resulted in reversal of hyperglycemia-induced dysregulation in vascular reactivity in bovine aortic endothelial cells. These results were paralleled in an in vivo experiment on the STZ-induced diabetic rat aorta [15]. Another study on STZ-induced diabetic rats demonstrated a moderate reduction in lesion size after 12 week glucosamine, suggesting a protective role [49]. Collectively, these experimental results suggest a possible role for the hexosamine pathway in diabetic vascular disease.

Advanced glycation end products

One of the most damaging effects of diabetes-induced hyperglycemia is the non-enzymatic reaction between glucose and proteins in the arterial wall leading to the formation of advanced glycation end products (AGE). The production of AGE is a physiological process, which is enhanced in the presence of elevated circulating glucose [45]. AGE formation (Fig. 1) is in part due to a succession of chemical reactions between glucose and proteins known as the Maillard or browning reaction, which is a non-enzymatic reaction between ketones/aldehydes and amino groups of proteins. During the Maillard reaction, reactive intermediate products methylglyoxal, 3-deoxyglucosone, and glyoxal are produced and play a role in the development of carbonyl stress and AGE formation [50].

As age advances, AGE accumulate continuously on vessel wall proteins and at a documented accelerated rate in DM, producing potentially atherosclerotic effects by interfering with the function of the endothelial cells (EC) that line the vasculature [51]. Glucose concentration appears to be the main determinant in AGE formation along with tissue redox potential. Permanent AGE formation disrupts molecular confirmation and alters enzymatic activity. Its detrimental effects in accelerating atherosclerosis are mainly two pronged and include: non-receptor-mediated mechanisms and receptor-mediated mechanisms [50].

Non-receptor-mediated mechanisms AGE modify intracellular proteins and gene transcription factors, nucleic acids, and macromolecules. They are able to modify extracellular matrix molecules by diffusing out of the cell, which may have implications in cellular signaling

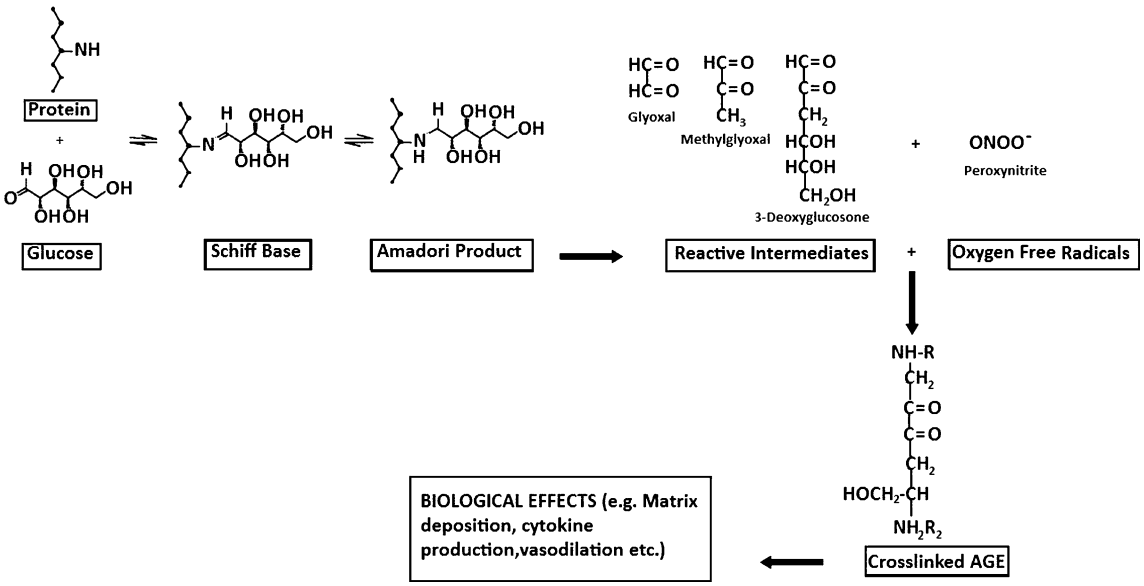

Fig. 1 Schematic representation of AGE formation by the Maillard process. The first step is a condensation reaction between an amino and carboxyl group (sugar) to form a reversible Schiff base adduct (early glycation product). Over the course of days to weeks, early glycation products ultimately result in the formation of stable, irreversibly bound AGE through intermediate molecular rearrangement and the production of Amadori rearrangement products. Carboxymethyl-lysine-protein adducts are the predominant AGE present in vivo [42]

processes. Moreover, they can diffuse into the blood and modify circulating proteins involved in the formation of atherosclerotic plaques [42]. Diabetes is known to accentuate changes in vessel wall elasticity associated with ageing and significant correlations have been recognized between the magnitude of AGE deposits and the severity of diabetic complications [52].

Furthermore, many in vitro and in vivo studies have indicated that AGE induce irreversible crosslinks in long-living matrix structural proteins, such as type 4 collagen, laminin, and fibrinectin, thereby reducing susceptibility to proteolysis and yielding fibrosis, loss of elasticity, and reductions in arterial compliance. Covalent and cross-linking modifications by glucose cause glycation of many structural and intracellular proteins, changing their confirmation, and damaging their function. In this respect, it is noteworthy that clinical studies have demonstrated increased levels of AGE on LDL obtained from diabetic patients and 4-fold higher AGE-Apolipoprotein B (ApoB) levels in diabetics [53]. Modification of ApoB renders the LDL particle more atherogenic by increasing its uptake by macrophages. Once internalized, foam cell formation is stimulated, which in turn induces recruitment of a host of inflammatory mediators and growth factors that promote atherosclerosis. Glycation also increases susceptibility of LDL to oxidative modification, which has serious atherogenic effects.

Receptor-mediated mechanisms The receptor for AGE, i.e., the transmembrane receptor proteins, which bind AGE (RAGE), have been identified using radio-labeled age proteins and have been demonstrated in monocyte-derived macrophages, endothelial, and smooth muscle cells [42]. By binding to AGE-modified proteins, RAGE initiates multiple cascades of signaling that activate protein kinase C (PKC), which induces pro-inflammatory cytokines and consequently inflammation, growth factor release, and fibrosis. AGE also alter cellular functions by binding to other receptors including the macrophage scavenger receptor, p60, p90, and galectin-3 ultimately effecting a disruption in cellular homeostasis [39]. Ligation of RAGE brings about activation of transcription factor nuclear factor κB (NFκB) that coordinates the inflammatory response [2]. Experimentation in animal models of both types 1 and 2 DM reveal that antagonism of the ligand–RAGE axis suppresses the key processes linked to acceleration of atherosclerosis and exaggerated neointimal expansion consequent to arterial injury [54]. The ligand families of RAGE accumulate in the vasculature in diabetes and appear to be enhanced in atherosclerotic lesions, as indicated by animal models [55]. The study of Park and colleagues [55] using apoE deficient diabetic mice clearly demonstrates the role of RAGE in the development and progression of vascular and inflammatory cell perturbation in the diabetic milieu, both of which are crucial in the pathogenesis of CAD. Homozygous deletion of apoE in the said mice made them more susceptible to atherosclerosis, which manifested with increased severity compared to controls. In the diabetic mice, development of atherosclerosis was more rapid and presented as complex lesions with extensive inflammatory infiltrate. Up-regulation of AGE expression was observed with concomitant presence of AGE at sites of vascular lesions. Most importantly, independent of glycemic status and lipid levels, blockage of AGE–RAGE interaction by a truncated soluble extracellular RAGE domain resulted in a significant suppression of lesions [55].

From the aforementioned considerations, it is evident that many aspects of diabetic complications are related to AGE. It has been proposed that blockade of RAGE may represent an effective target for therapeutic intervention in diabetes and its cardiovascular complications. As such, the anti-AGE drug, LR-90 was shown to inhibit AGE formation, cross-linking and exert other atheroprotective properties in the rat aorta, during in vivo pharmaceutical intervention of the Maillard reaction [56].

Protein kinase C activation

Protein kinase C is a family of enzymes comprised of at least 12 isozymes involved in a diverse range of cellular responses including proliferation, contractility, hypertrophy, signal transduction, growth factor transcription and apoptosis [57]. Glucose-induced activation of PKC has been documented in many experimental models of diabetes, both in animals, and humans [58]. Activation of PKC in diabetes has been postulated to cause cardiovascular dysfunction by stimulating extracellular matrix production, activation of the inflammatory response by cytokine expression and leukocyte adhesion, loss of vascular reactivity, increased endothelial permeability, BM thickening, and angiogenesis [50].

Hyperglycemia increases levels of the PKC activator diacylglycerol (DAG) in endothelial cells by de novo synthesis from dihydroxy acetone phosphate (DHAP) and glyceraldehyde-3-phosphate as given by:

$$\mathrm{DHAP} \xrightarrow{\mathrm{NADH}\rightarrow\mathrm{NAD}^{+}} \mathrm{DAG} \rightarrow \mathrm{PKC}$$

Indirect activation may also be brought about by RAGE or polyol pathway activation. Once activated, the pro-atherogenic effects of PKCs are myriad and include dysregulation of vascular reactivity, impairment of fibrinolysis through increased plasminogen activated inhibitor-1 (PAI-1) expression and elevation of redox stress by effects on NADPH oxidases. In the presence of hyperglycemia, PKC is also known to up-regulate adhesion molecules in

endothelial cells (EC), thus providing impetus to the inflammatory response [59].

In vascular smooth muscle cells (VSMC), PKC activation has been shown to influence mitogenesis, DNA synthesis, and growth factor receptor turnover [60]. PKC activation increases the expression of transforming growth factor-β (TGF-β) that governs extracellular matrix formation [41]. It is also particularly noteworthy that peroxisome proliferators activated receptor-γ agonists, which are used in diabetes therapy prevent hyperglycemia-induced activation of PKC in VSMC and EC [59]. Inhibition of PKC, especially the β1/2 isoform with ruboxistaurin (PKCβ isoform selective inhibitor) has been reported to prevent or normalize many vascular abnormalities in diabetes including endothelial dysfunction, which is the initiating event in atherosclerosis [61].

Activation of the aforementioned pathways in DM and their collective involvement in vascular damage has been the subject of over 13,000 articles, since 1966 [62], suggesting strong pathogenetic links. It has been hypothesized that the four pathways are linked to a common upstream event that is triggered by hyperglycemia. Research in this area has been pioneered by Brownlee and colleagues [63] who demonstrated that overproduction of reactive oxygen species (ROS) by the mitochondria occurs under high-glucose conditions increasing oxidative stress. In the hyperglycemic milieu, the role of oxidative stress has been highlighted as causative in the development of accelerated atherosclerosis [42, 64] and is an important concept that merits discussion.

Oxidative stress In normal physiological conditions, 95% of oxygen consumed, undergoes tetravalent reduction by the cytochrome oxidase system, forming water and ATP. The remaining 5% oxygen is univalently reduced and four electrons are added one at a time. This process leads to the formation of a variety of highly reactive ROS [65]. ROS include free radicals, such as superoxide ($O_2^{-\bullet}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), hydroperoxyl ($HRO_2^{-\bullet}$) as well as non-radical species, such as hydrogen peroxide (H_2O_2) and hydrochlorous acid [66]. Should the generation of ROS exceed their neutralization by antioxidants (such as glutathione), oxidative "stress" results, and producing pathological consequences, such as damage to proteins, lipids, and DNA. Relevant to macroangiopathy, ROS modify endothelial function by peroxidation of membrane lipids, transcription factor activation, and by interfering with NO bioavailability [67]. Importantly, there is evidence that superoxide and peroxynitrite are important mediators of pancreatic cell death, and thus, might serve as pathogenic factors precipitating the disease itself [68].

In the literature, there is a general consensus that the production of ROS is elevated in diabetic individuals and several clinical experiments have documented increases in levels of oxidative stress markers such as oxidized low density lipoprotein (ox-LDL), lipid hydroperoxides, plasma and urinary F_2-isoprostane and DNA-damage products like 8-hydroxydeoxyguanosine (8-OHdg) [69, 70]. DNA damage as quantified by measuring levels of 8-OHdg in mononuclear cells showed greater oxidative damage to DNA in diabetic patients compared to controls and an approximately 2-fold increase in plasma oxidative stress [70]. A reduction in antioxidants has also been reported in diabetic patients, and an observation that was mirrored in STZ-induced diabetic rats where levels of the antioxidant superoxide dismutase were 50% lower compared to controls [71]. There are multiple sources of increased oxidative stress in DM of which hyperglycemia-driven mitochondrial overproduction of ROS is particularly significant. It has been put forward as being the primary biochemical abnormality that is the initiating event in the activation of the four pathways mentioned previously [62]. An overview of the supposed mechanism is as follows.

Endothelial cells are unable to regulate glucose transport in response to external changes in glucose concentration. Therefore, in DM, EC exposed to hyperglycemia have an increased substrate flux through glycolysis and the tricarboxylic acid cycle. Mitochondrial overproduction of superoxide by way of increased electron donors (NADH and $FADH_2$) into the electron transport chain causes an escalating voltage gradient across the inner mitochondrial membrane and concomitant increased ROS (superoxide) production ensues. It has been determined that increased ROS production by the mitochondria induce DNA strand breaks, which in turn activates the nuclear DNA repair enzyme poly(ADP-ribose) polymerase (PARP). As a consequence of modification by PARP the activity of the key glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GADPH) is substantially inhibited resulting in the upstream accumulation of glycolytic intermediates, such as glyceraldehyde-3-phosphate and fructose-6-phosphate. The net result is increased superoxide production, glyceraldehyde-3-phosphate dehydrogenase (GADPH) inhibition and activation of the four pathways of hyperglycemic damage. This mechanism can be further explicated by the schematic representation as shown in Fig. 2.

Another major source of oxidative stress in diabetes is through glucose auto-oxidation. In vivo experiments indicate that in the auto-oxidation of glucose, free radical production is increased, yielding superoxide anion, and hydrogen peroxide [65]. Moreover, decreased efficiency of inhibitory scavenger systems, such as glutathione, vitamin C, and vitamin E result in overproduction of precursors to ROS. Experimental evidence suggests hyperglycemia may compromize antioxidant defences as such as glutathione and reduce vitamin E in diabetic patients [42]. A total of 40–50% lower vitamin C levels in tissue and plasma were

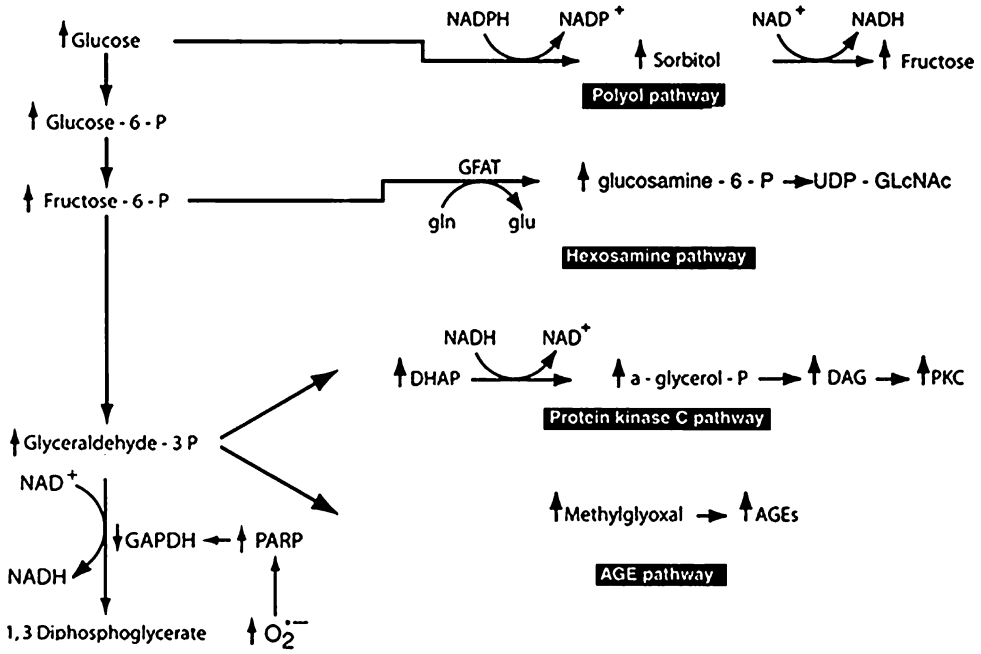

Fig. 2 Overproduction of superoxide by the mitochondria activates four major pathways of hyperglycemic damage by inhibiting GADPH adapted with permission from [63]. The upstream glycolytic metabolite glyceraldehyde-3-phosphate is a precursor to methylglyoxal and diacylglycerol. Hence, increased levels activate the AGE pathway and PKC pathway, respectively. Further upstream, levels of the glycolytic metabolite fructose-6-phosphate increase, which in turn increases flux through the hexosamine pathway. Finally, GAPDH inhibition has also been found to increase intracellular glucose and flux through the polyol pathway [62, 63]. Conversely, the 4 pathways have a common denominator in oxidative stress as each one singularly acts to increase ROS levels

observed in diabetic subjects compared to euglycemic controls [72]. Glycation of antioxidants results in their gradual inactivation and levels of the glycated antioxidant Cu–Zn-superoxide dismutase (CuZNSod) have been reported in the erythrocytes of diabetic patients [73].

On purely statistical grounds, poor glycemic control might appear to increase the risk of diabetic macroangiopathy, but closer observation reveals a particularly weak correlation, especially in the case of type 2 DM that is often preceded by or associated with the metabolic syndrome. As per epidemiological studies, coexistence of both DM, and the metabolic syndrome increased the prevalence of CAD to 19.2% of the population over 50 years of age [74].

Substantial controversy exists surrounding the effects of glycemic management on cardiovascular outcomes in patients with diabetes mellitus. Moreover, despite strong associations between increasing glycemia and cardiovascular risk it has not been clearly demonstrated that lowering of blood glucose improves cardiovascular outcome in patients with type 2 diabetes mellitus [75]. While reduction of hyperglycemia had been shown to discernibly reduce microvascular complications, some studies report that diabetic patients displayed a higher risk for developing macrovascular complications, in spite of achieving normoglycemia [43] Thus, it could be inferred that hyperglycemia is necessary but not "sufficient" [76] to cause diabetic macroangiopathy and its adverse sequelae, therefore implicating non-glucose mediated mechanisms, such as insulin resistance and dyslipidemia.

Insulin resistance

Insulin signaling elicits a vast variety of biological responses, including regulation of carbohydrate, lipid and protein metabolism, DNA replication, protein synthesis, and enzyme activity. Although the term "insulin resistance" (IR) could refer to any of the pleiotropic effects of insulin, the classical definition applies to resistance to insulin-mediated glucose disposal. IR is the primary biochemical abnormality in most cases of type 2 DM and a common feature of type 1 DM. Even without overt DM, IR is known to accelerate CAD. Its onset gives a way to a slow decline in blood glucose regulation later accompanied by compensatory hyperinsulinemia that persists until β cell dysfunction occurs. By the same token, the view that insulin resistance may be the initial lesion leading to Type 2 diabetes cannot be marginalized [77].

At the cellular level, insulin binds to its receptor, which is a transmembrane tyrosine kinase. Post-receptor signaling is achieved by two pathways, namely, the Phosphoinositol-3 kinase/Akt pathway (PI(3)K/Akt) pathway, which plays a major role in insulin-stimulated glucose uptake and the

mitogen-activated protein kinase (MAPK) cascade, which involves phosphorylation of adaptor protein Shc and activation of Ras (Fig. 3). The latter mainly contributes to the nuclear and mitogenic effects of insulin [78] by phosphorylation of various nuclear transcription factors.

Insulin resistant states, such as impaired glucose tolerance, type 2 DM, and to a certain extent, obesity are characterized by a severe defect in IRS-1 activation. Resultant hyperglycemia stimulates insulin production and release giving rise to compensatory hyperinsulinemia. However, in the state of IR, the mitogenic MAPK pathway remains intact and when excessively stimulated (by compensatory hyperinsulinemia), cause detrimental pro-atherogenic effects, such as increased production of inflammatory mediators and growth factors, increased VSMC proliferation, and reduced fibrinolysis [79].

Both insulin resistance and hyperinsulinemia have received much attention as possible etiologic factors in the development of atherosclerotic heart disease wherein it is suggested that magnitude of IR positively correlates with severity of CAD. In order to cite an example, the studies of Pyörälä et al. [80] showed that insulin concentrations were significantly related to coronary heart disease in men.

IR and hyperinsulinemia may accelerate CAD by exerting direct effects on the vessel wall. In general, the principal effects of insulin are anti-thrombotic, anti-inflammatory, anti-atherogenic, and are likely to be damaged in the state of IR. Many of the regulatory effects of insulin on EC depend on its ability to produce vasoconstrictor and vasodilatory agents and in normal conditions insulin has a protective vasodilatory action mainly governed by NO production. In vitro experiments have demonstrated insulin-stimulated production of the vasodilator NO in cultured EC, and an effect that was blunted by the PI(3)K inhibitor Wortmannin [8]. Similarly, insulin has been demonstrated to blunt the response of several agents of vasoconstriction while itself producing endothelin-1 (a potent vasoconstrictor), mainly through the MAPK pathway [81]. Insulin is also a mediator of VSMC differentiation and quiescence through the PI(3)K pathway. In

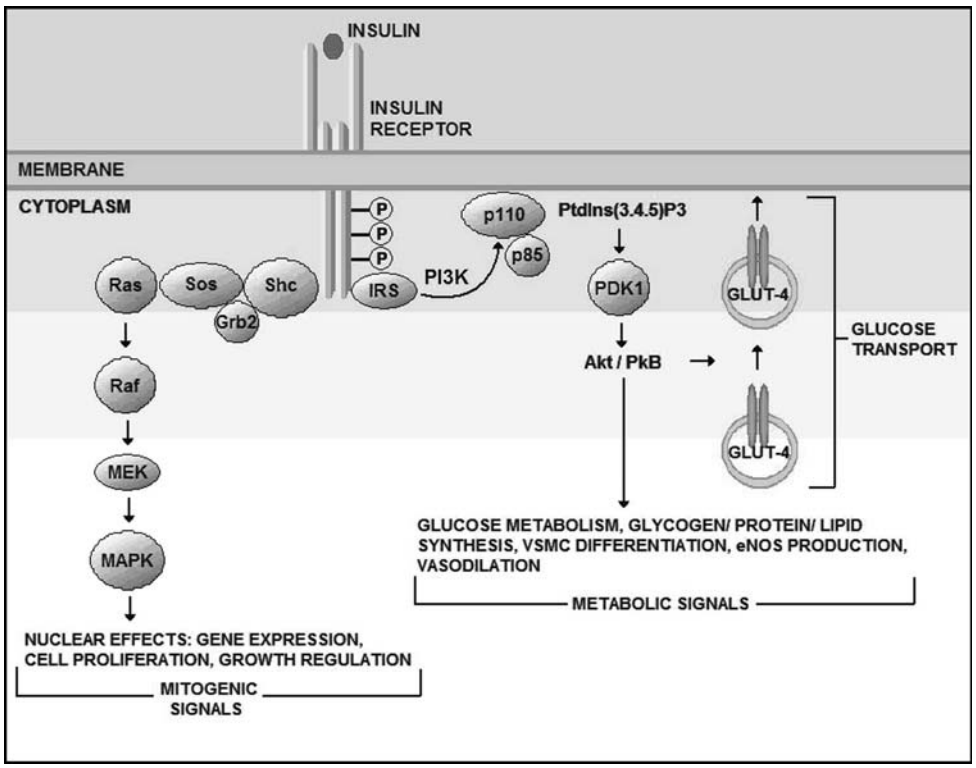

Fig. 3 Simplified illustration of insulin signaling. Insulin binding and subsequent receptor autophosphorylation (P) leads to tyrosine phosphorylation of intracellular protein substrates activating two major branching pathways. A key downstream effector of the IRS pathway is Akt/PKB activated through the action of heterodimeric (p85/p110)P1(3)K and formation of Ptd(3,4,5)P3 and PDK1. Apart from modulating glycogen, protein, and lipid metabolism and nitric oxide generation, PI(3)k and Akt stimulate glucose uptake into cells by influencing the translocation of glucose transporter GLUT4. The mitogenic effects of insulin post-receptor signaling (such as cellular growth, proliferation, and survival) are brought about by sequential activation of several signaling molecules and cytoplasmic protein kinases collectively termed the Ras/MAPK signaling cascade. (*IRS* Insulin receptor substrate, *PKB* Protein kinase B, *PI(3)K* Phosphoinositide 3-kinase, *PDK1* 3-Phosphoinositide-dependent protein kinase-1, *Ptd(3,4,5)P_3* Phosphatidylinositol-3,4,5-triphosphate, *NO* Nitric oxide, *MAPK* Mitogen-activated protein kinase.)

conjunction with other atherogenic growth factors, such as platelet-derived growth factor (PDGF), insulin facilitates proliferation, and migration of VSMC.

By inducing loss of vascular reactivity, impairment of normal vasomotion, and blood flow through reduced NO bioavailability, IR plays a major role in the onset of endothelial dysfunction that precedes atherosclerosis. It has been shown that endothelial dysfunction is closely correlated with the presence of insulin resistance. In IR, the cumulative effect of PDGF and a blunted insulin response is atherogenic. As observed in the rat aorta, IR, and hyperinsulinemia further stimulate VSMC proliferation and arterial wall lipid deposition by LDL-receptor activity up-regulation and production of growth factors. It also switches on the genes that are involved in connective tissue formation, an important part of the atherosclerotic lesion [82]. It is especially noteworthy that diabetic and insulin resistant individuals present with high insulin levels as proper glycemic control in type 2 diabetic patients usually requires $\geq$100 units of insulin per day [83]. At this concentration, insulin may exhibit an atherogenic potential that has been a cause of concern in recent years.

Supporting evidence notwithstanding, the direct relation between insulin resistance and atherogenesis has not been fully established. It has been suggested that the major effect of insulin on atherosclerosis is probably mediated through its effect on other cardiovascular risk factors including dyslipidemia, hypertension, and impaired fibrinolysis rather than by a direct effect of insulin. In support of this school of thought, even studies that have found a relation between IR and atherosclerosis and between fasting insulin level and CAD have not accounted for all conventional risk determinants (such as fibrinolytic factors) [84]. Also, in vitro experiments generally use insulin at supraphysiological concentrations, which raises the possibility that the Insulin like Growth Factor-1 (IGF-1) receptor may intercede. Further complexity arises from the multifarious interplay of genetic determinants of IR and others that independently regulate lipid metabolism and the biology of the vascular wall. Therefore, it might be prudent to exercise a certain degree of discretion in the interpretation of experimental results.

Insulin resistance has been implicated as an indirect cause of atherogenesis by promoting the development of dyslipidemia. Early on in the course of IR, free fatty acids (FFA) increase owing to a loss of the suppressive effects of insulin on lipolysis in adipocytes. In the literature, the role of elevated circulating FFA has been emphasized as a key factor that links IR and dyslipidemia. Also, as over 80% of people with type 2 diabetes are obese, a significantly greater amount of FFA is released from an expanded adipose tissue mass [85]. Coupled with disturbed insulin signaling in adipose tissue, the overall effect is abnormal lipid metabolism and a pro-atherogenic phenotype. In addition to elevated hepatic very low density lipoprotein (VLDL) synthesis (as a result of increased substrate (FFA and glucose) delivery and resistance to the inhibitory effect of insulin on VLDL secretion), there is a reduced rate of VLDL removal by peripheral tissues due to resistance of lipoprotein lipase to insulin [86]. Action of the enzyme lipoprotein lipase is rate-limiting for the clearance of triglyceride-rich lipoproteins. Increased circulating FFA stimulate the assembly and secretion of VLDL from the liver and hence in the plasma, ultimately causing accumulation of VLDL in the blood vessel wall. A correlation exists between plasma levels of VLDL and deposits of cholesterol in the vessel wall, mediated by levels of the formation of small dense LDL. Subsequently, cholesterol binds to proteoglycans in the ECM and contributes toward plaque formation. Additionally, elevations in the levels of FFA activate PKC β and δ, reducing glucose transport by phosphorylation of the insulin receptor substrate [8]. Free fatty acids and obesity also contribute to the development of IR and it has been established that insulin sensitivity is modulated by adiposity. FFA reduces insulin sensitivity in muscle by inhibiting insulin signaling, glucose transport/phosphorylation, glycogen synthase, and pyruvate dehydrogenase [78].

In addition to releasing FFA, visceral body fat is known to have important endocrine implications in DM with fat cells producing a variety of adipocytokines that are crucial to the regulation of insulin signaling and action. Some examples include TNF-alpha, Il-6, leptin, and adiponectin [86]. By an elegantly designed set of experiments, Brownlee and colleagues [87] tested the hypothesis that increased FFA (in IR and DM) have a central role in diabetic macrovascular disease. This proposition was supported by experimental evidence from cell culture and animal models and was not replicated in microvascular endothelial cells. In arterial endothelial cells, it was found that increased FFA flux and oxidation by the mitochondria causes an overproduction of mitochondrial ROS by the same mechanism as described previously in for glucose. Moreover, as was the case with hyperglycemia, elevated ROS production through increased FFA resulted in the activation of NFκB, PKC, and hexosamine pathway, with concomitant increase in AGE production [87].

From these considerations, it may be reasonable to assume that IR characteristic of type 2 DM (and a common manifestation of type 1 DM) is a dominant influence in the development and progression of CAD.

Dyslipidemia

In general, the lipid hypothesis for the accelerated development of atherosclerosis in DM stems from the

premise that dyslipidemia is central to the process of obstructive vascular disease [52]. Although diabetes is considered to be primarily a disorder of glycemic control, dyslipidemia is common in diabetes and MS and is a known risk factor for coronary atherosclerosis [88, 89]. The risk of atherosclerosis in obesity and other lipid abnormalities depends on the balance between atheroprotective ApoAI (which promotes cholesterol transport to the liver for excretion), ApoB (which is the major component of cholesterol-rich LDL), and VLDL particles. As discussed earlier, IR in type 2 DM and the MS results in increased levels of FFA by the liver. Through a sequence of events involving cholesterol ester transfer protein (CETP), VLDL becomes cholesterol rich, whereas high density lipoprotein (HDL) is transformed into smaller and denser particles, susceptible to the action of lipoprotein lipase. VLDL's and circulating FFA also result in the activation of cytokines and transcription factors that encode genes for a number of atherogenic mediators. Concentrations of small dense LDL particles are also increased by the action of CETP and these particles have reduced affinity for the LDL-receptor, thus extending their duration in the plasma. This mechanism is strongly atherogenic as small dense LDL particles infiltrate the arterial wall and contribute to plaque formation.

Diabetic dyslipidemia presents as hypertriglyceridemia, elevated small LDL particles, and low HDL levels, also known as the "lipid triad" [49]. Data from the Framingham study show that twice as many diabetic compared to non-diabetic individuals have low plasma levels of HDL-C and elevated triglycerides. Diabetic patients are observed to have more severe CAD and a 3-fold increase in MI [90] compared to non-diabetics owing to small, dense LDL particles [88], which have been observed to demonstrate increased atherogenic potential due to increased susceptibility to oxidation and increased ability to cross the endothelial barrier [91]. In the subendothelial space, LDL undergoes oxidation and results in the generation of ROS by macrophages, EC, and VSMC. Oxidized LDL (ox-LDL) is cytotoxic to EC as it impairs endothelial NO-dependent vasodilation. Modified LDL is chemotactic for leukocytes [92] and also induces macrophage proliferation at the subendothelial space, thereby promoting the inflammatory response. Along with initiating endothelial dysfunction, diabetic dyslipidemia is also a determinant in the growth and maturation of atherosclerotic lesions. In support of this statement, from experiments conducted on transgenic mice it was deduced that diabetic dyslipidemia (increased triglyceride-rich VLDL) contributed largely to the progression of advanced atherosclerotic lesions, advanced plaques, and intralesional hemorrhage [93, 94].

The pathophysiology of atherosclerotic heart disease in DM

Pathobiology of plaque formation: an overview

Atherosclerosis is the central pathological mechanism in CAD. The onset of CAD starts as early as the first decade of life and progresses eventually to the formation of atherosclerotic plaques that gradually narrow the arterial walls. Unstable or obstructive plaques lead to the clinical manifestations of atherosclerosis and CAD [95]. The working definition of atherosclerosis as appropriately described by Hayden and Tyagi [96] is "a systemic dysfunctional endothelial, focal occurring, chronic inflammatory, fibro-proliferative, prothrombotic, angiogenic, multifactorial disease of the arterial intima caused by the retention of modified low-density lipoproteins, hemodynamic, and reductive-oxidative (redox) stress."

Atherosclerosis is the result of formation of multiple plaques within the arteries by a process commonly referred to as atherogenesis. The initiating event in plaque formation has been the subject of much speculation and three major hypotheses are most widely accepted. These include the "response to injury" hypothesis, the "response to retention" hypothesis, and the "oxidation" hypothesis [8]. The response to injury hypothesis, propounded by Russell Ross in 1973, focuses on endothelial dysfunction as a trigger for chronic inflammation associated with atherosclerosis [97]. The response to retention hypothesis proposes that the sub-endothelial retention and subsequent accumulation of lipids by the ECM is the key event in the initiation of atherogenesis. This theory was further extended in 1994 to accommodate the oxidation hypothesis, which stipulates that ox-LDL in the vessel wall originates an immune response and an inflammatory stimulus, which ultimately exacerbates lesion formation [98]. Following the initial insult, redox signaling induces adhesion factors to recruit monocytes and platelets to adhere to the area of injury. Monocytes differentiate to macrophages which internalize ox-LDL and form foam cells. Aggregation of foam cells appear like a "Fatty streak," which is a subendothelial lipid and macrophage deposit presenting as a yellow, raised area on the luminal surface of the artery [99].

The inflammatory process is further propagated by the death of the foam cells and lesion continues to develop as VSMC migrate into the intima. During this process intimal "cushions" present at branching points of the affected arteries as a result of intimal thickening by edematous patches and an increase in ECM molecules, VSMC collagen, etc. Continued ingestion of excess ox-LDL particles by macrophages and monocytes results in inflammatory cytokine and growth factor release which perpetuates a

vicious cycle of inflammation, lipid deposition, and growth, and forming the "atheroma" that precedes the atherosclerotic plaque. The atherosclerotic plaque is characterized by the thickening of the arterial intima and is characterized by: (1) elevated numbers of smooth muscle cells, macrophages, and lymphocytes, (2) cholesterol deposits and (3) dense layers of connective tissue matrix. Additionally, calcification may be observed at the periphery of more advanced lesions. On the basis of gross morphology, plaques can be distinguished into fibro-lipid and fibrous plaques [100]. Although both plaques are subendothelial and result in compensatory expansion of the muscular layer of the vessel wall, the former consists of a fibrous cap covering the atheromatous core. Necrosis occurs in areas of the core making it an accumulation of tissue debris, proteoglycans, and collagen with lipid laden cells. The fibrous plaque contains eosinophilic collagen fibers and hematoxylinophilic precipitates of calcium. Gradual occlusion of the artery by plaques can lead to conditions, such as stable angina.

Glagov and colleagues [101] first demonstrated that the arterial wall can remodel itself by increasing its external diameter to accommodate the thickening plaque without significant lumen narrowing. However, in spite of compensatory artery enlargement, over time, atheromatous plaques may rupture, and cause stenosis of the artery [102]. In advanced-stage coronary atherosclerosis, the wall of the atherosclerotic artery shows thickening, reduction in lumen diameter and loss of elasticity and stiffening due to calcification deposits. Plaque rupture is the hallmark of acute coronary events and exposure of the thrombogenic core to the coagulation cascade leads to thrombus formation [92]. These complications may cause either partial or total occlusion of the lumen leading to the adverse outcomes of CAD, namely, infarction, stroke, claudication or in some cases subtotal occlusion, and unstable angina [103].

Features of coronary atherosclerosis in DM

Both type 1 and type 2 DM are associated with diffuse coronary disease characterized by a greater lipid core burden and inflammatory infiltrate [104] and increased number of fissures that may lead to lumen reduction and a greater risk of ischemic events [105]. The "Pathological determinants of Atherosclerosis in Youth" study examined atherosclerosis in the right coronary artery in subjects ranging from 15 to 34 years, who died of external causes. Based on HbA_{1c} values, if was determined that right coronary arteries exposed to hyperglycemia were twice as likely to have fatty streaks on >5% of the intimal surface and three times more likely to have macroscopic raised lesions compared to control [106]. In other similar investigations of atherectomy specimens, the cell-rich and necrotic areas were found to be increased in de novo lesions in persons with diabetes [104].

Morphological features of coronary atherosclerosis in diabetic patients are studied mainly by Computed Tomography (to determine calcium deposit), Ultrasound (to determine intimal medial thickness), and intravascular ultrasound. Although expensive and invasive (as in the case of ultrasound), these methods offer potential for earlier detecting, staging, and establishing disease progression. Employing these methods, pathologic, angiographic and in vivo studies converge that diabetics present with a significantly higher number of diseased vessel segments, greater proportion of concentric lesions, maladaptive remodeling, and a tendency for acute disruption [105]. Excess coronary artery calcification (CAC) is apparent in type 1 diabetes compared with general non-diabetic control populations as established by The Coronary Artery Calcification in Type 1 Diabetes Study [107]. Furthermore, in a subset of patients with unstable angina, angioscopic examination revealed that plaque ulceration and intracoronary thrombus formation are more common in diabetics compared to non-diabetic patients [108].

At this point, it is necessary to bear in mind that a comprehensive investigation of the nature and distribution of coronary atherosclerosis in the diabetic setting has not been accomplished as many of the studies mentioned above have been event or procedure driven [108]. As a result of these selection biases, the results might not wholly characterize atherosclerotic lesions in the total diabetic population.

Mechanisms of accelerated coronary atherosclerosis in DM

The published literature represents a large body of evidence that plausibly links DM with a marked increase in atherosclerosis and CAD (Fig. 4). Atheroma formation and development of atherosclerosis in diabetic individuals includes a complex interplay of aforementioned factors, such as hyperglycemia, hyperlipidemia, oxidative stress, and insulin resistance. In conjunction with alterations in coagulation and fibrinolysis, autonomic, and cardiac changes, these diabetic traits are associated with greater extent and severity of atherosclerosis as DM appears to be a multiplier of risk factors [109]. The pathophysiology of atherosclerosis in DM is characterized by (1) dysfunction of the endothelium, VSMC, and platelets, (2) a prothrombotic and proinflammatory state, and (3) impaired collateral formation. A discussion of these key features in diabetes-accelerated atherosclerosis follows in the context of plaque formation, growth, and ultimately rupture.

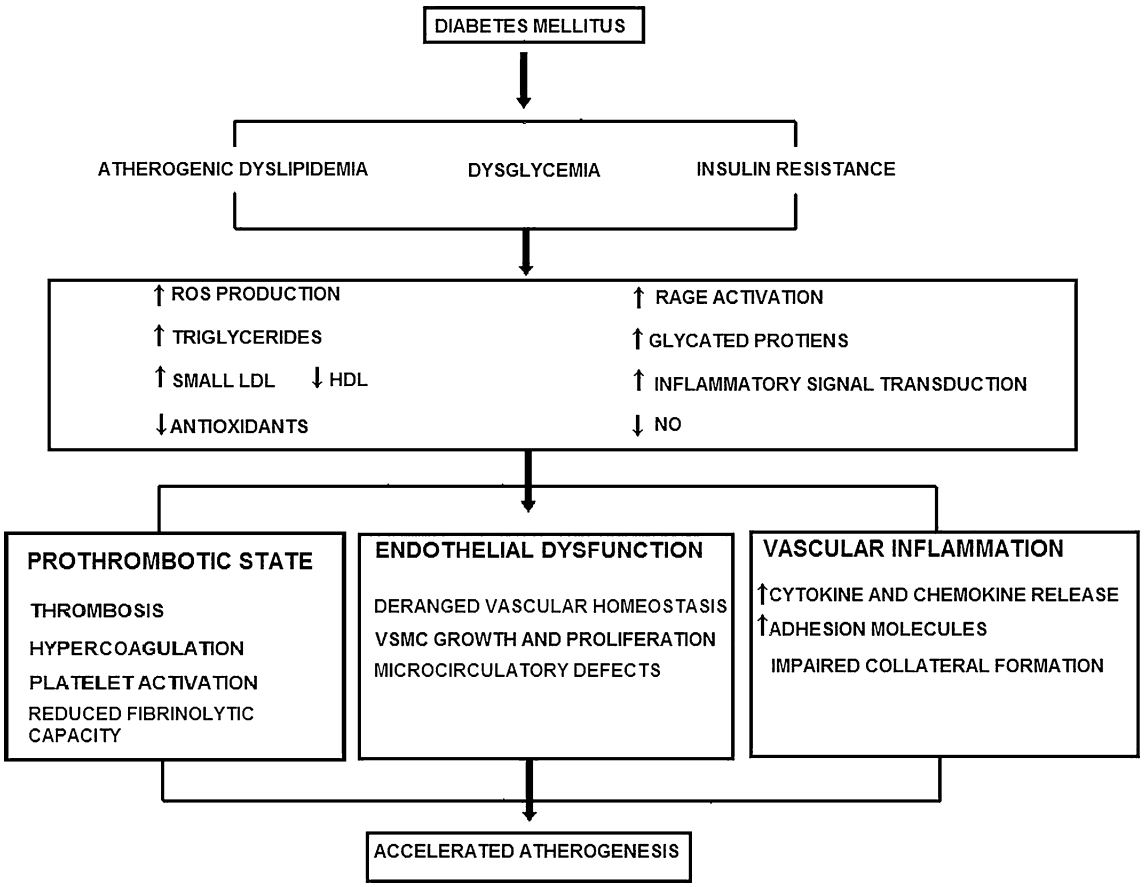

Fig. 4 Metabolic abnormalities in DM stimulate a cascade of events that initiate endothelial dysfunction and promote atherosclerosis. (Mechanisms explained in the text. Symbols indicate alterations relative to controls, ↑ increased ↓ decreased; *ROS* Reactive oxygen species, *NO* Nitric oxide, *LDL* Low density lipoprotein, *HDL* High density lipoprotein, *RAGE* Receptor for advanced glycation age products.)

Initiation of lesion formation

Endothelial physiology

The endothelium is central to the barrier against atherosclerosis. It lines the internal lumen of all the vasculature, serving as a metabolically active interface between circulating blood and VSMC. The endothelial lining functions as a receptor–effector organ with a vast array of highly regulated mechanisms essential for normal vascular function and overall homeostasis that are governed by chemical mediators.

Through autocrine, paracrine, and endocrine mechanisms, the healthy endothelium regulates blood flow, nutrient delivery, coagulation, thrombosis, and inflammation [110]. Although the principal effect of endothelium stimulation is vasodilation, the endothelium also affects the functions of VSMC, platelets, leukocytes, and macrophages [43]. It intervenes in platelet activation, inflammation, VSMC proliferation, and migration, altogether functioning to keep atherogenesis and thrombogenesis in check. In doing so, the healthy endothelium independently regulates vascular homeostasis by maintaining a delicate balance between agonistic and antagonistic substances [111]. In the present context, one of the most important substances produced by the endothelium is NO, synthesized through the action of the enzyme nitric oxide synthase (NOS). NO crosses the endothelial intima, reaches the smooth muscle tissue, and causes vasodilatation (smooth muscle fiber relaxation) by regulation of cytosolic Ca^{2+} through a mechanism that degrades Guanosine triphosphate (GTP) to release cyclic guanosine monophosphate (cGMP) [112].

Shear stress caused by increase in blood velocity is the most powerful stimulant for NO production and it activates and opens Ca^{2+} activated K^{+} channels that hyperpolarize the endothelial cell, activating endothelial NOS (eNOS). In order to digress slightly, shear stress is also significant in the context of plaque formation and clinical outcomes as plaques tend to localize in areas where shear stress is low

(<6 din/cm^2) and NO release is blighted whereas high shear stress (>70 din/cm^2) causes damage to the plaque and induces platelet aggregation [113]. Thus, hemodynamic factors play an important role in the initiation and progression of atherosclerotic plaques.

Apart from regulation of vasomotor tone, much experimental evidence has led to the concept that NO also plays a critical role in vascular homeostasis, neuronal, and immunological functions. Basal NO production promotes a continual vasodilation and regulation of blood pressure [109]. Beyond its known vasodilating capabilities, NO has several pleiotropic functions in the vasculature. Normal NO function might be perceived as a vital defence mechanism to the pathogenesis of atherosclerosis due to inhibitory effects on platelet aggregation and adhesion, leukocyte adhesion, VSMC proliferation, and activation of thrombogenic factors. For example, NO counterbalances the stimulatory effect of Vascular Endothelial Growth Factor (VEGF) on expression of adhesion molecules and expresses the inhibitor of nuclear factor κB (NfκB) [114]. In vitro NO has been observed to mitigate monocyte adhesion to the endothelium by decreasing the expression of leukocyte adhesion molecules and cytokines [115]. Additionally, NO has been shown to mediate DNA synthesis, mitogenesis, and proliferation of VSMC [116]. Thus, owing to its vasodilator, anti-platelet, anti-proliferative, anti-inflammatory, and permeability decreasing properties, the bioavailability of NO is a prerequisite to vascular health.

Endothelial dysfunction

The process of atherosclerosis begins at the endothelium and dysfunction of the endothelial lining is at the crux of atherosclerotic lesions that present throughout the course of the disease. Being at a rather strategic position between the circulating blood and VSMC, the endothelium is a mediator of and at the same time may be regarded as susceptible to CVD. While it might be difficult to assess cause and effect, CVD risk factors alter the capacity of EC leading to "dysfunction." That established, endothelial dysfunction is defined as the failure of the vascular endothelium to achieve its normal role of vasodilatation and preservation of vascular homeostasis [117], paving the way for pathological inflammatory processes, such as atherosclerosis and vascular diseases, such as CAD. Endothelial dysfunction is associated with late-stage adverse outcomes and precedes morphological vascular changes. Nitenberg et al. [118] first confirmed loss of endothelial vasoreactivity in the coronary arteries. Since, then, various invasive and non-invasive techniques have shown that patients with CAD and increased cardiovascular risk factor profile present with a dysfunctional endothelium [95]. Although the exact mechanism of endothelial dysfunction in humans has not been elucidated, several studies point to reduced NO bioavailability and maintain that tight glycemic control can improve endothelial reactivity [119]. In this regard, an overview of endothelial dysfunction as an outcome of hyperglycemia, its immediate biochemical sequelae, and loss of vascular reactivity is presented as follows.

Diabetes is associated with diffuse "endotheliopathy" [96], which pertains to abnormalities in the dysregulation of the lumen of vessels mainly brought on by hyperglycemia and its immediate biochemical sequelae [76]. As all of the changes occurring in atheroma formation are subendothelial, EC damage, and loss of protective NO effects are very likely the first steps in atherosclerosis. By a series of cellular events related to the endothelial nitric oxide synthase (eNOS) reaction the anti-atherogenic, protective effects of the healthy endothelium are disrupted. In EC, NO is synthesized by the following reaction:

$$\text{L-arginine} \xrightarrow[\substack{\text{eNOS}\\ \text{BH}_4}]{\text{NAD(P)HNAD(P)HOxidase}} \text{NO} + \text{L-citrulline} + \text{NAD(P)}^+$$

eNOS is responsible for most of the vascular NO produced and a functional eNOS oxidizes its substrate L-arginine to L-citrulline and NO by a reaction that utilizes NADP coenzyme and O_2. This normal function of eNOS requires the presence of the substrate L-arginine, and the cofactor (6*R*)-5,6,7,8-tetrahydro-L-biopterin (BH_4), which is a potent reducing agent. In DM, a process known as eNOS "uncoupling" occurs wherein eNOS switches from NO production to generation of superoxide anion. Underlying factors in eNOS uncoupling are a depletion of L-arginine and BH_4, both of which have been reported to occur in DM, mostly as a result of increased oxidative stress [119]. Increased ROS formation (specifically peroxynitrite) causes the uncoupling of eNOS by oxidizing its cofactor BH_4 to inactive BH_2, ultimately resulting in the net production of superoxide instead of NO. Endothelial dysfunction can be reversed by dietary supplementation of BH_4, which "recouples" eNOS and enhances NO production and this effect has been demonstrated in in vivo models [120]. Furthermore, the pro-oxidant state in DM and concomitant increase in ox-LDL limits L-arginine availability in EC, which contributes to eNOS uncoupling and defective NO production.

In DM, vascular reactivity is further impaired by production of vasoconstrictive agents, such as prostanoids and endothelin. Hyperglycemia, hyperinsulinemia, RAGE ligation, PKC activation, and ox-LDL all bring about increased endothelin-1 (ET-1) activity and production of endothelin. Along with dysregulation in vascular tone, ET-1 brings about salt and water retention, stimulates the

renin–angiotensin system, promotes inflammation, and has mitogenic effects in VSMC [121]. Endothelin-1 is the most potent vasoconstrictor known and its significance in CAD has been demonstrated wherein it was reported that the majority of basal vascular tone in atherosclerotic coronary arteries is mediated by endothelin-1, which accounts completely for the vascular tone at stenoses [122]. The effects of hyperglycemia on NO production and vascular homeostasis have been well demonstrated by in vitro experiments. For example, incubation of bovine EC with high concentrations of glucose was shown to inhibit eNOS expression and leads to diminish NO bioavailability [123]. Similarly, in vitro studies on the rabbit aorta exposed to hyperglycemia showed increase in vasoconstrictor prostanoids [124].

Dyslipidemia and IR further potentiate the action of hyperglycemia in endothelial activation and dysfunction. The role of IR in endothelial dysfunction is particularly noteworthy as eNOS expression and activity are mediated through the insulin signaling pathway that involves activation of PI(3)K. Hence, in DM, IR causes a significant reduction in NO synthesis through diminished signaling through the PI3K/Akt pathway. Reduced NO production allows increases in VEGF-induced expression of adhesion molecules and monocyte recruitment, which is a pro-atherogenic process. The net result of decreased NO production and increased expression of vasoconstrictor agents is diminished NO bioavailability, loss of vascular reactivity, and the protective effects of NO that cumulatively conspire toward pro-atherogenic consequences.

As discussed previously, hyperglycemia causes altered redox states by altering the $NADH/NAD^+$ ratio, dysregulation of PKC, and sorbitol accumulation with concomitant generation of AGE's. These pathways result in elevated levels of ROS (particularly $O_2^{-\bullet}$), in EC and VSMC and have been implicated directly in endothelial damage [125]. Glucose transport occurs by facilitated diffusion in endothelial cells and when exposed to high concentrations of glucose in vitro, EC have been found to increase the production of extra-cellular matrix components such as collagen and fibronectin, pro-coagulants, such as von Willebrand factor (vWF) and exhibit decreased proliferation, migration, and apoptosis. AGE formation leads to accumulation of ox-LDL molecules, which indirectly impair EC function. Hyperglycemia, in the presence of insulin resistance may also be a direct effector of increased levels of growth factors and cytokines, such as VEGF and TGFβ that stimulate the EC, causing endothelial activation, and dysfunction [114]. Endothelial activation and dysfunction results in morphological vascular changes including weakening of intercellular junctions by overexpression of proteases, such as μ-caspase, disappearance of capillary endothelium, and altered protein synthesis [117]. Increased levels of triglycerides and low HDL associated with diabetic dyslipidemia have also been associated with endothelial dysfunction. Increased FFA in dyslipidemia and IR cause damage to the endothelium by the production of ROS and PKC activation. In the healthy endothelium leukocyte adhesion is maintained at a minimum, but dyslipidemia in DM promotes leukocyte adhesion and ox-LDL in the arterial intima leads to the release of phospholipids that activate endothelial cells. The activated endothelium is associated with increased adhesion molecule production, leukocyte–EC interaction, and glycosylation of proteins, such as lipoproteins and clotting factors. These aspects cause pro-atherogenic effects, such as an increase in pro-inflammatory gene activity and growth factor up-regulation, platelet aggregation, metaloprotease expression, and the sustenance of an environment favorable to thrombogenesis, all of which are features of the exaggerated inflammatory response observed in DM.

Plaque growth and maturation

Inflammation

Atherosclerosis is today recognized as a low grade inflammatory and ultimately thrombotic process, contrasting the traditional view of passive accumulation of lipids within artery walls. Inflammation, which is accompanied by the production of numerous inflammatory biomarkers, such as cytokines, acute phase proteins, adhesion molecules, transcription factors, interferons, chemokines, etc., is a central mechanism contributing to the progression of CVD, and may be involved in the triggering of myocardial ischemia [126] (Fig. 5). Several studies have demonstrated an association between increased expression and plasma concentration of these biomarkers and current or future overt CAD [127].

A review of inflammation in atherothrombotic disease provides convincing evidence of the significant and independent role of coronary (and systemic) inflammation in the instigation and progression of atherothrombosis against a background of traditional risk factors [128]. A close relation is also present between the aforementioned biomarkers and glucose metabolism abnormalities leading to a speculated "common soil" inflammatory basis for CAD and diabetes that advocates common molecular mechanisms between inflammatory and insulin signaling pathways. At the core of this supposition lays the fact that dysfunction of both inflammatory and insulin signaling pathways cause IR and endothelial dysfunction that synergize to predispose for cardiovascular disease [129]. Along with the recognized involvement of inflammatory processes in type 1 DM, a large body of evidence suggests that in type 2 diabetic individuals, features of inflammation

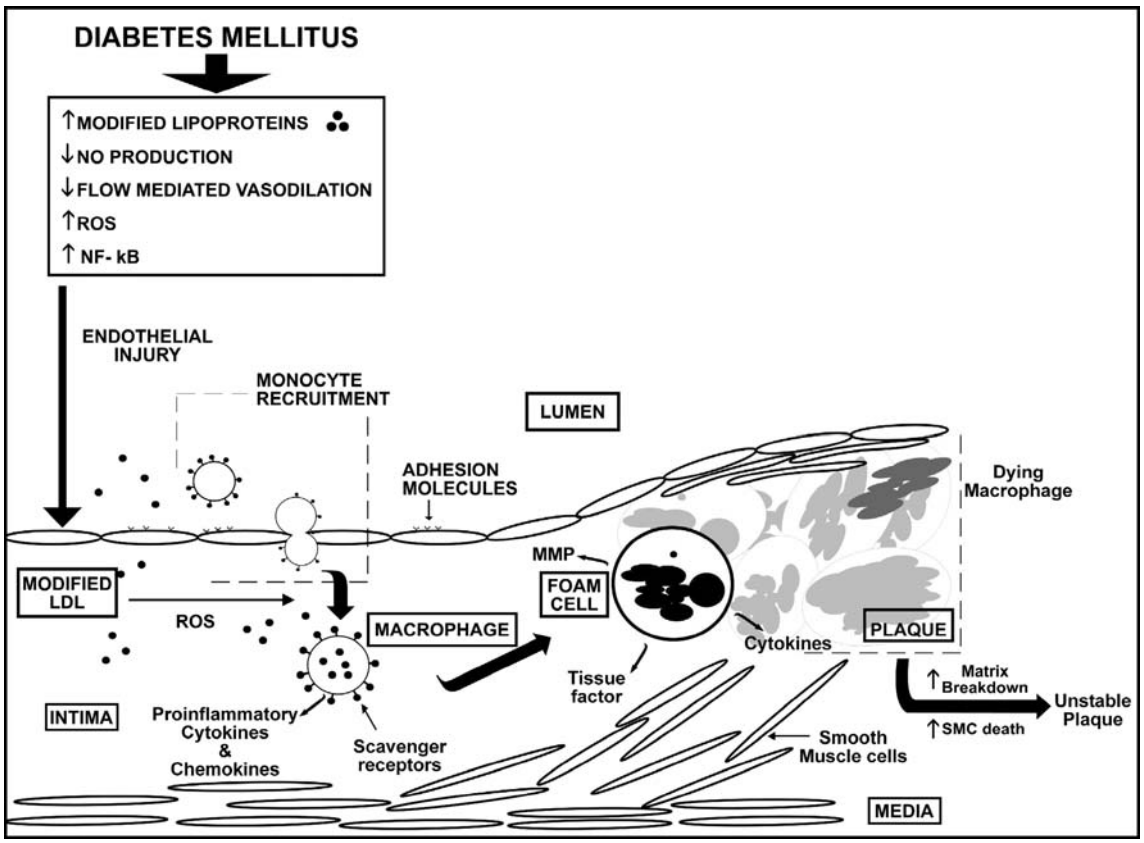

Fig. 5 Schematic representation of inflammatory mechanisms involved in plaque formation in diabetes-accelerated atherosclerosis. The processes in question are grossly simplified, but illustrate the prominent interactions between inflammatory infiltrate and components of the vascular wall in an approximate temporal sequence from left to right. In DM, multiple atherosclerotic risk factors enhance oxidative stress in vascular cells, activate signaling molecules, such as NFkB and induce endothelial dysfunction characterized by decreased NO synthesis and increased entry of modified circulating lipoproteins into the vessel wall. In response to inflammatory activation, the dysfunctional endothelium elaborates an up-regulation of adhesion molecules (VCAM, ICAM) facilitating adherence and transendothelial migration of leukocytes into the tunica intima mediated by a chemoattractant gradient. In the intima, inflammatory mediators, such as M-CSF, can augment expression of scavenger receptors that aid detection and internalization of modified lipoprotein particles by macrophages ultimately forming foam cells and initiating lesion formation. In the process of lesion evolution, lymphocytes and resident vascular wall cells secrete cytokines and growth factors that promote migration of VSMC into the intima and penetration through the elastic lamina and collagenous matrix of the evolving plaque. MMP secreted by macrophages degrade the ECM, which is a key determinant of plaque stability. In conjunction with the action of gamma interferon produced by T-lymphocytes, which limits collagen production, the overall outcome is a vulnerable plaque susceptible to rupture defined by extensive inflammatory infiltrate, a lipid core, and a thin fibrous cap. (Symbols indicate alterations relative to controls, ↑ increased; ↓ decreased. *ROS* Reactive oxygen species, *MMP* Matrix metaloprotease, *NFkB* Nuclear factor κB, *LDL* Low density lipoprotein.)

are apparent years before onset of the disease, suggesting a cause and effect mechanism [128]. Further evidence of the pathogenetic role of inflammation in the onset of diabetes has been provided by clinical trials demonstrating the efficacy of anti-inflammatory agents in preventing or delaying the onset of DM in high risk subjects [130].

As highlighted in the illustration, each step in the pathophysiology of atherosclerosis, i.e., from endothelial dysfunction to plaque rupture and clinical events is characterized by participation of inflammatory constituents. Metabolic abnormalities that characterize the state of impaired glucose tolerance in DM initiate and further intensify humoral, cellular and subcellular responses in coronary arteries that lead to vascular inflammation. As such, hyperglycemia, oxidative stress and oxidized lipids, FFA, AGE and PKC activation along with decreases in NO bioavailability result in increased activation, physiological modulation, and pathological alteration of transcription factors that mediate immune and inflammatory responses, of which NFκB is most notable. It activates a variety of target genes influencing pathological alteration in the vessel wall, including cytokines, chemokines, and leukocyte adhesion molecules, as well as genes that regulate cell proliferation and mediate cell survival [131–133].

In DM, decreased NO, high levels of LDL-C, Ang II, and oxidative stress also elicit an increase in the

concentration of adhesion glycoproteins including integrins like VCAM-1, ICAM-1; selectins, such as E-selectin and P-selectin and receptors for oxidized lipid particles, such as leptin like ox-LDL receptor (LOX-1) [92]. This represents an early phase in atheroma formation where the recruitment of circulating inflammatory cells is facilitated by enhanced expression of adhesion molecules expressed by EC. Serum concentrations of VCAM-1 and ICAM-1 have been found to be elevated in subjects with type 2 DM and CVD [126] and circulating concentrations of ICAM have been correlated with risk of CHD [92]. Infiltration of monocytes, macrophages, and T cells into the arterial intima results in the production of several other growth factors and inflammatory mediators, ultimately promoting, and sustaining growth of fatty streaks, which evolve into full-fledged atherosclerotic plaques (Fig. 5). As expected, diabetic patients exhibit plaques with a "hyperinflammatory" composition. Additionally, increases in AGE production as a result of poor glycemic control in DM provide a strong antigenic stimulus for inflammatory mediators in atherosclerotic plaque, and further amplifies the release of inflammatory cytokines. Cytokines, such as Interleukins (IL), tumor necrosis factors (TNF), interferons (IFN), colony stimulating factors (CSF), and transforming growth factors (TGF) have been demonstrated to play a central role in atherosclerotic processes [102] and in DM, are influenced by IR and glucose intolerance to produce global pro-atherosclerotic activity. Prominent examples of cytokines dysregulated by the effects of diabetes are IFN-γ, TNF-α, and the interleukins. Interleukins are critical, early mediators of inflammation, and elevated concentrations of some interleukins are associated with increased risk of developing diabetes [129]. Serum levels of the cytokines TNF-α and IL-6 are significant predictors of future cardiovascular events in healthy subjects as well as diabetics along with the pro-inflammatory cytokine IFN-γ, which is of the strongest predictors of cardiovascular death as well as of development of type 2 diabetes [134].

All together, the spectrum of cell responses elicited by cytokines is diverse. Acting in concert, they could be hypothesized to induce β-cell damage and apoptosis by infiltration of the pancreas thereby precipitating the onset of DM. Pro-inflammatory cytokines increase oxidative stress, alter normal vasomotor responses, and increase the migration and adhesion of immune cells to the endothelium [135]. Recently, Lin and colleagues reported increased cytokines (Il-6 and TNF-α), chemokines, and adhesive molecules in the adventitia of STZ-induced diabetic swine as compared to controls and have suggested the role of a documented increase in oxidative stress, AGE production, and NFkB activation in mediating the inflammatory response [136]. These considerations reinforce the proposition that inflammation is cornerstone in the pathophysiology of DM-associated atheroma progression, provoking plaque growth, progression, destabilisation, and rupture. The balance between pro- and anti-inflammatory agents has emerged as a major determinant of plaque stability [101].

VSMC dysfunction

Whereas the early events in atheroma formation involve primarily altered endothelial cell function, the subsequent evolution of the atheroma into an atherosclerotic plaque involves focal thickening of the intima with an increase in VSMC migration, and extra-cellular matrix development [137]. Diabetes stimulates atherogenic activity of VSMC, which play integral parts in plaque maturation and progression [43]. Following formation of the fatty streak, VSMC from the underlying medial layer migrate into the intimal lesion in response to chemoattractant signaling by PDGF, IGF-1, and thrombin [93]. PDGF, secreted by activated macrophages, is chemotactic for VSMC and is found to be overexpressed in human models of atherosclerosis. On migration to the intimal layer, VSMC replicate and lay down a complex extracellular matrix, which makes the plaque less likely to rupture, mainly through the production of collagen, elastin, and proteoglycans, which form the fibrous cap and contributes to the stability of the plaque. This fibrous cap surrounds a lipid rich core in advanced plaques. Autopsy studies show that lesions, which have disrupted and caused fatal thrombosis, have fewer VSMC as do advanced atherosclerotic lesions in diabetic patients [117].

Insulin is an important mediator of VSMC differentiation and quiescence via signaling though the PI(3)K pathway. In the diabetic state, IR results in loss of the anti-atherogenic, protective effect of insulin on the VSMC, and signaling through the unaffected MAPK pathway resulting in cell migration and proliferation [89]. This effect has been replicated in vitro by the application of Wortmannin to inhibit insulin action in human arterial VSMC [138]. The impact of hyperglycemia on VSMC function is still a subject of some debate and its contribution toward VSMC accumulation in atherosclerotic lesions in vivo is said to be primarily indirect, i.e., mediated by characteristics, such as increased sensitivity to circulating factors, such as cytokines, endothelial dysfunction, matrix production, and macrophages. Modification of LDL by AGE is particularly important in this context as it induces VSMC migration and apoptosis of cells in atherosclerotic lesions [43]. Additionally, in VSMC exposed to high-glucose concentrations, activation of PKC, and NFκB occurs, which in turn elevates ROS production adding to oxidative stress.

Plaque rupture

Plaque stability

The risk of acute coronary events is dependent upon tendency for plaque rupture as opposed to degree of occlusion of the lumen and acute coronary syndromes, such as arterial occlusion, stroke, or MI mostly involving soft, modestly stenotic plaques. The same principle is reiterated in the diabetic state, wherein ultrasonography studies showed that diabetics typically exhibit diffuse and extensive CAD with mostly modest occlusion of coronary arteries (severity of stenosis ranging from <25% to <75%) and very few subjects exhibited severe CA occlusion >95%) [92].

Irrespective of glycemic status, most deaths due to coronary events in the susceptible are a result of atherosclerotic plaque disruption, thrombosis and release of atheromatous, fibrous and necrotic materials, and various types of activated immune cells. The stability of the plaque is a function of factors, such as the relative proportion of lipids, inflammatory infiltrate, connective tissue, and the configuration of the plaque in relation to shear stress. In general, unstable coronary artery plaques present with substantial lipid core, extensive inflammatory infiltrate, reduced VSMC and collagen, increased neovascularization and a thin fibrous cap narrowing the vessel lumen by <50% [139]. The fibrous cap is an important determinant of plaque stability.

The strength of the fibrous cap and its tendency for rupture rely on the balance between collagen deposition and degradation. Activated macrophages in the plaque may promote plaque rupture by secreting metaloproteases, cathepsins, and collagenases. Furthermore, cytokine expression impedes the assembly of collagen by VSMC that fortifies the plaque. The metabolic abnormalities in DM promote an environment highly conducive to rupture of the vulnerable plaque. As described in Fig. 5, atherosclerotic lesions from diabetic patients are characterized by higher apoptosis of VSMC, higher NFκB activation, and metaloprotease levels along with a lesser interstitial collagen content [140]. Increased ROS levels in diabetes as a result of monocyte infiltration through EC and ox-LDL phagocytosis in foam cells enhance the production of MMP. Elevation in MMP activity in DM due to increased synthesis and reduced breakdown by tissue inhibitors of MMP result in increased matrix degradation and destabilization of the plaques, which may trigger thrombus formation [119].

Impaired platelet function

Platelets are integral to the regulation of vascular homeostasis and therefore, abnormalities in platelet formation may exacerbate the progression of atherosclerosis and consequences of plaque rupture. In addition to being chief effectors of hemostasis by formation of blood clots, platelets are rapidly deployed to sites of injury or infection and intervene in the inflammatory process by secretion of cytokines and chemokines [141]. Damage to blood vessel walls and proaggregatory stimuli like thrombin, norepinephrine, and collagen cause circulating platelets to bind collagen with surface collagen-specific glycoprotein Ia/IIa receptors with concomitant release of agents, such as ADP, serotonin, platelet-activating factor, and vWF, which are stored in granules and thromboxane A_2 (TXA_2). These, along with other components, such as coagulation pathway factors and growth factors induce the activation and aggregatory action of other platelets [23].

Diabetes results in platelet hyper-reactivity, which manifests as increased aggregation and adhesion. Increased platelet adhesiveness and aggregability in response to common platelet-activating stimuli, such as adenosine and collagen has been demonstrated in diabetic rats [142]. Also, patients with diabetes display increased platelet-surface expression of glycoprotein Ib and Gp IIb/IIIa, which mediate increased platelet-vWf factor and platelet–fibrin interaction [121]. The primary defect in the platelets exposed to high-glucose concentrations may be a disorder of calcium homeostasis since intraplatelet calcium regulates platelet shape change (spherical to stellate), secretion, and aggregation [122, 143]. Other factors causing impairment of platelet functions are reduced membrane fluidity, which is related to membrane phospholipid to cholesterol ratio, increased arachidonic acid metabolism and thromboxane A2 synthesis, and elevated glycation of platelet membrane proteins. Increased platelet aggregation that is demonstrated by diabetic patients in early diseased states may be positively correlated with the onset of CVD and may increase tendency for thrombus formation [117].

The most thoroughly studied inhibitors of platelet activation are the anti-aggregants NO and PGI_2, which are continuously produced in healthy vessels and limit the growth of the platelet plug to the area of vascular injury. PGI_2 and NO prevent platelet aggregation and adherence to endothelium and their synthesis is further amplified in the vicinity of aggregating platelets [144]. Their effector systems activate adenylate cyclase and cAMP that alter counteract the effects of increased cytosolic levels of calcium by alterations in phosphorylation state, enzymatic activity, and structural properties of proteins, which inhibit platelet activation. Also, the same processes hinder release of granules that would lead to activation of additional platelets and the coagulation cascade. Therefore, defective endothelial production of NO and PGI_2 in the diabetic state results in loss of containment of platelet aggregation, which might be compounded by IR and increased production of

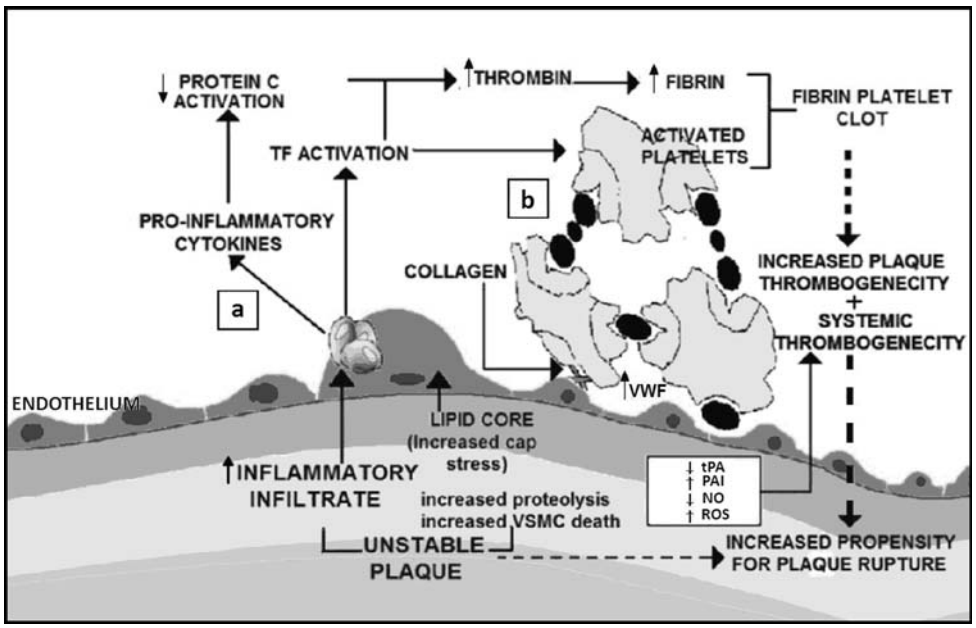

Fig. 6 Illustration of the processes sustaining a prothrombotic state in DM. Diabetes mellitus accelerates the development of atherothrombosis by reducing fibrinolytic capacity and mediating the shift to a procoagulant state characterized by increased inflammatory mediators, PAI-1, plasma fibrinogen, vWF, and thrombin. (a) Endothelial dysfunction and NO deficit in DM impair endogenous inhibition resulting in enhanced platelet susceptibility to aggregate in response to agonists released from the inflamed endothelium. T-lymphocytes induce increased expression of the potent procoagulant tissue factor by plaque macrophages, highlighting the fundamental relationship between arterial inflammation and atherothrombosis. (b) Post-plaque rupture, activation of circulating platelets by adhesion proteins, such as vWF and collagen results in aggregation. TF released from the lipid core and subendothelial matrix activates the coagulation cascade resulting in thrombin generation and fibrin formation. The overall effect is the sustenance of an environment conducive to generation and persistence of coronary thrombi and the precipitation of acute coronary syndromes. (Symbols indicate alterations relative to controls, ↑ increased; ↓ decreased. *TF* Tissue factor, *vWF* von Willebrand Factor, *tPA* Tissue plasminogen activator, *PAI-1* Plasminogen activator inhibitor-1, *NO* Nitric oxide, *ROS* Reactive oxygen species.)

platelet activators, such as vWF and thrombin [49]. Altogether, these changes in the platelets and the endothelium increase the likelihood of thrombosis (Fig. 6).

Disturbances in coagulation and fibrinolysis

Clinical consequences of plaque rupture depend not only on plaque anatomy and platelet function but also on relative balance of procoagulant and anti-coagulant activity in the blood and on the vulnerability of the myocardium to arrhythmias. Following rupture of an atherosclerotic plaque, the coagulability of the blood is an important factor in determining the extent, and impact of resulting thrombus formation.

Diabetes is also associated with a procoagulant state and can exacerbate activation of the coagulation cascade. It is associated with an increase in the number of coagulation factors, such as tissue factor and factor VII, and a decrease in endogenous anti-coagulants, such as anti-thrombin III and protein C [121]. Lower antithrombin levels result in increased thrombin and impaired fibrinolytic activity whereas protein C deficiency results in platelet hypercoagulability by the enhanced activation of factors V and VIII. A possible mechanism of the procoagulant state that accompanies DM is via formation of AGE and increased oxidative stress, which result in lower levels of anticoagulants, such as tissue factor and antithrombin III [117]. Also, it has been documented that in diabetic individuals, the intrinsic pathway of the coagulation cascade is enhanced, along with increased levels of kallikrein, factors XII, XI, V III, and vWf [145]. Although no direct evidence exists to link activation of the coagulation cascade and endothelial injury in human models of DM, it is not entirely unfeasible that repeated activation of the coagulation cascade and production of fibrin fragments lead to overstimulation of EC and endothelial dysfunction.

The procoagulant state in DM is further sustained by a disturbance in the balance of prothrombotic and fibrinolytic mechanisms that occur in endothelial dysfunction. Fibrinolysis is stimulated by plasminogen activation and inhibited by antagonists, such as PAI-1. In DM, endothelial dysfunction compounded by inflammation, increased PAI-1 levels in the plaques/plasma further aggravate the injury to the vessel by promotion of thrombosis. Epidemiological studies have demonstrated that hemostatic disturbances,

such as excessive fibrinogen levels and low fibrinolytic activity (excessive PAI-1, reduced tissue plasminogen activator (tPA) levels), are associated correlated with re-infarction and degree of CAD. Elevated levels of PAI-1 have been shown in patients with type 2 DM at risk for atherothrombotic complications in atheromatous lesions and non-atheromatous arteries [146]. It is noted that with subsequent cardiac events fibrinogen levels and impaired fibrinolysis are more common in diabetic patients than in non-diabetic control subjects, but the underlying cause has not been definitively identified. Many factors, both genetic and environmental including age, smoking habits, BMI, fat distribution, sedentary lifestyle, and microalbuminuria may influence the parameters of hemostasis and act as confounding factors [23].

The role of hyperinsulinemia in an impaired fibrinolysis cannot be discounted. Insulin results in an increased endothelial expression of PAI-1 by EC causing a shift in the balance of coagulation and fibrinolysis toward the latter. This provides impetus to the accumulation of fibrin within the arterial walls and consequently to the progression of atherothrombotic disease. Also, high plasma levels of thrombin–antithrombin III complexes have been observed in type 2 DM patients [146], conceivably as a result of AGE and oxidative stress increments, circuitously resulting in excess fibrin production. Another potent trigger of the coagulation cascade is tissue factor (TF) that mediates arterial thrombosis [74]. Of relevance to the present discussion, Sambola et al. [147] have identified that improvement of glycemic control in diabetic patients causes significant reductions in TF activity and blood thrombogenicity. In precis, diabetes-associated platelet dysfunction, procoagulability and impaired fibrinolysis strongly predispose towards thrombotic occlusion post plaque rupture (Fig. 6).

Neovascularization abnormalities

The heterogeneous nature of presentation of DM is especially evident in the context of neovascularization. The disease is associated with exaggerated angiogenesis in the retina leading to diabetic retinopathy and in the vessel wall, potentially promoting atherosclerotic plaque destabilisation. On the other end of the spectrum, insufficient angiogenesis is also a symptom of the disease and gives rise to complications, such as diabetic skin ulcers.

Diabetes-induced dysregulation in neovascularization is also evident from the standpoint of atherosclerotic heart disease. Within the vulnerable plaque, an important factor that has received noticeably less consideration in the literature is the increased vascularization within the intima and the media following hypoxia and ischemia during thickening of the vessel wall. In DM, the multiple metabolic derangements associated with diabetes increase redox stress and ischemia, which induces the vasa vasorum (Vv) to undergo angiogenesis in response to Hypoxia inducible factor-1 (HIF-1) and VEGF stimulation [96]. Adventitial derived Vv that promotes intraplaque angiogenesis, which in the setting of the vulnerable plaque may be perceived as a double-edged sword. Neovessels are the body's natural response to injury and serve to nourish the outward remodelling intima. On the other hand, it also provides nutrients to inflammatory and smooth muscle cells [74] thereby contribute to the maturation of the plaque by the "response to injury" mechanism as previously described. Moreover, new vessels created by the process of angiogenesis lack smooth muscle cells, are fragile and highly prone to rupture [148]. Diffuse microangiopathy associated with DM further augments the already heightened inflammatory response during the extravasation of RBC plasma membranes. This series of events gives rise to intraplaque hemorrhages, which play important roles in transforming stable into unstable plaques by provision of angiogenic as well as antigenic stimuli, amplifying both neovascularization, and intraplaque inflammatory responses.

The scope of neovascularization correlates with the extent of inflammatory cells and the link between inflammation and accelerated angiogenesis is gaining importance in the context of the vulnerable plaque [149]. In DM Vv angiogenesis is further induced by mediators of inflammation, such as tissue ACE, tissue factor, cytokines, and growth factors, all of which are acknowledged angiogenic factors [150]. In a study that evaluated microvessel and inflammatory cell content in atherosclerotic plaques in diabetic patients, increased microvessel content, macrophages, T-lymphocytes, and intraplaque hemorrhages (IPH) were found in diabetic compared to control subjects and the authors have speculated that plaque composition may increase the risk of rupture in diabetic atherosclerosis [151]. Indeed, IPH activate inflammatory macrophages at the shoulder of the plaque causing secretion of MMP and collagenases ultimately resulting in erosion, fissuring, and rupturing of the plaque.

The severity of macrovascular complications in DM may be partly due to profound impaired collateralization of vascular beds [152], but the mechanisms that underlie neovascularization defects in DM remain somewhat intangible. Both clinical and experimental DM provide evidence of anomalous structural and functional characteristics of the coronary collateral circulation [153]. Diabetic patients are known to exhibit inadequate collateral vascular formation in response to ischemia, which increases cardiovascular morbidity and mortality rates [154]. Abaci et al. [155] demonstrated that the development of coronary collateral vessels is significantly reduced in

patients with diabetes mellitus in response to ischemia by retrospective assessment of the prevalence of coronary collateral vessels in 205 coronary angiograms from diabetic patients with different severities of coronary artery disease.

Angiogenesis, defined as true capillary formation out of pre-existing ones, can only partly contribute to enhanced tissue perfusion. Functional collateral vessels are formed out of pre-existing ones by a process that is best described by the term "arteriogenesis," i.e., growth of preexisting arterioles (collateral vessels or anastomoses) [147, 156]. Either repetitive or chronic epicardial blockage synonymous with the advanced stages of atherosclerosis, incite development of coronary collaterals [96] that preserve myocardial function and viability by reducing myocardial ischemia and functional deficit [153]. Shear stress is commonly accepted to be the initial driving force in collateral formation supported by the fact that it is usually distant to the area of tissue ischemia and in proximity to occluded vessels [156]. In contrast to angiogenesis, which leads to the de novo formation of capillaries, arteriogenesis describes vascular growth in diameter, beginning with small (20 μ) minimally functioning arterioles, and remodelling to form larger arterioles 1–2 mm in diameter that are capable of greater blood supply to ischemic tissue [96, 150]. A number of cellular events precipitate increase in vascular diameter including proliferation of endothelial progenitor cells and VSMC, actions of cytokines, chemokines, inflammatory infiltrate, and MMP [148]. Waltenberger and colleagues reported an increase in monocyte infiltrate in developing collaterals, thereby highlighting the importance of the monocyte in collateral formation [157]. Activation of monocytes using either MCP-1 or lipopolysaccharide has been shown to promote arteriogenesis through the release of cytokines and growth factors [158]. Endothelial dysfunction in DM results in the up-regulation of monocytes within the wall of the growing collateral and this process is further potentiated by cytokines, such as Basic fibroblast growth factor, MCP-1, and VEGF-A in particular [156].

Both in vitro and in vivo experiments have shown that VEGF is a major mediator of neovascularization and it plays a central role in developmental blood vessel formation and regulation of hypoxia-induced tissue angiogenesis [159]. Two high-affinity VEGF tyrosine kinase receptors that are almost exclusive to EC have been identified: fms-like tyrosine kinase 1 (flt-1, also known as VEGF-R1), which is the principal receptor involved in VEGF signaling and fetal liver kinase 1 (flk-1), or VEGF-R2 [54]. The angiogenic action of VEGF in EC is effected mainly by signaling through the PI3K–Akt–eNOS axis. In the vessel wall, VEGF signaling contributes to arteriogenesis by direct actions on the endothelial layer which increases MCP-1 and induces up-regulation of adhesion molecules. Predictably, monocyte migration is increased and monocytes/macrophages further promote VEGF release by producing growth factors, such as Fibroblast growth factor 2 (FGF2) [156]. Post-cardiac ischemic events, a significant up-regulation in VEGF expression occurs in the myocardium, which is assumed to aid development of collateral vessels in the advanced stages of coronary atherosclerosis. Increases of cardiac VEGF expression in human and rodent hearts after MI has been observed in various experimental studies making VEGF gene transfer an important therapeutic avenue to induce re-vascularization after critical ischemia [160].

Diabetes mellitus is identified as a negative predictor of collateral formation and this may be due to the inability of monocytes to migrate toward a gradient of VEGF-A. In diabetic individuals, this impaired response seems to be secondary to a signal transduction defect downstream of VEGFR1/Flt-1 within the monocyte [156]. The inflammatory reaction, a crucial process in collateral formation is thus diminished. Furthermore, arteriogenesis appears to be NO-dependent to a certain extent and therefore, in DM, reduced NO could result in an impaired vasodilation and permeability, which disrupts the initiation and progression of remodelling collateralization. Other mechanisms affecting collateralization in DM may be the ECM transformation occurring as a consequence of AGE production. Finally, PAI-1 excess in DM may also impair monocyte migration indirectly through impaired plasmin synthesis and MMP inactivation [155].

From the above considerations, it appears as if a "vascularisation paradox" [96] occurs in diabetes where angiogenesis is induced and arteriogenesis is impaired. The molecular mechanisms underlying these vascularization abnormalities in DM remain largely speculative. An interesting hypothesis has been put forward to explain the defiance of a common molecular mechanism, involving the mainly the action of VEGF and its receptors Flk-1 and Flt-1 [150]. This hypothetical scheme of events extends experimental evidence put forward by Sasso et al. [153], wherein increased VEGF expression was found in the myocardium of diabetic patients with advanced coronary artery disease. In the same subjects they reported no concomitant increase in the expression of Flk-1 and Flt-1 receptors and down-regulation of VEGF-dependent intracellular signaling.

In order to explain the neovascularization paradox in the diabetic heart, it is proposed that impairment in the activation of Flk-1 (which is the main determinant of VEGF-mediated angiogenic signaling) as observed from the aforementioned study leads to increased levels of circulating VEGF in an attempt to compensate for the perceived deficiency of VEGF signaling [153]. This

down-regulation and desensitization of receptors and high circulating VEGF levels cause increased permeability of vascular structures throughout the body. For example, VEGF levels are significantly elevated in ocular fluid in diabetic retinopathy the retina and induce a local inflammatory response resulting in capillary sprouting [161]. A similar process might take place in the arterial wall, thereby promoting capillary sprouting and plaque destabilization. At the same time, the lack of Flk-1 activation in endothelial cells and abnormal VEGF-dependent activation of monocytes impair the arteriogenic response that requires monocyte recruitment and monocyte and endothelial cell migration and proliferation. In addition, VEGF/Flk-1 signaling is an important step for bone marrow release of circulating endothelial progenitor cells (EPC), which are recognized as putative progenitors for neovascularization. Alterations in EPC function or number are hypothesized to be involved in the pathogenesis of vascular complications and experimental evidence exists to show that poor coronary collateral development in DM may be related to low levels of circulating EPC [152]. Also, EPC alterations were demonstrated in patients with CAD and have been linked with risk factors for atherosclerotic disease [162]. The processes instrumental in causing a reduction of EPC in diabetes are a matter of debate although several mechanistic hypotheses have been propounded. These include weak bone marrow mobilization, exposure to unfavorable vascular environment characterized by increased oxidative stress, and apoptosis or senescence of EPC [152].

Coexistence of microvascular and macrovascular heart disease in DM

The higher prevalence and severity of CAD in DM is well established, but the role of coronary disease in the adverse prognosis of DM is controversial. The excess cardiac mortality is analogous to the excess of coronary atherosclerosis in some studies, while others have reported mortality after acute coronary events, such as MI with left ventricular dysfunction as opposed to widespread CAD, presence of risk factors or end-organ diseases [163]. Moreover, several groups have demonstrated a mitigation of the excess mortality following MI in diabetics by administration of ACE-inhibitors, without modifying the well known metabolic hallmarks of the disease (such as IR) [164]. Thus, in explanation of the excess cardiac vulnerability to DM, several other mechanisms have been proposed, including microcirculatory dysfunction, reduced metabolic resistance to ischemia, inflammatory and immune disorders, etc. Apart from disorders of the microcirculation, a further discussion of the causal factors in excess cardiac mortality is outside the remit of this review.

While CAD is the most common form of cardiac disease in DM, diabetic cardiomyopathy, presenting as non-ischemic heart failure is an important cause of morbidity and mortality in diabetic patients [20]. Diabetic cardiomyopathy refers to a disease of the diabetic myocardium that causes a wide range of structural abnormalities eventually leading to LV hypertrophy and diastolic and systolic dysfunction or a combination of these. It has a multi-factorial etiology, putative factors being increased myocardial fibrosis causing loss of ventricular compliance and stiffening, autonomic neuropathy, structural changes in collagen, alterations in myocardial energy metabolism, alterations in contractile proteins, and perhaps of greatest consequence in the present discussion, microvascular dysfunction [18]. Alterations in the structure and function of the microcirculation have been reported in diabetic cardiomyopathy. Structurally, arterioles, capillaries and venules in the diabetic myocardium exhibit a diffuse microangiopathy, and hyaline arteriosclerosis. Other structural alterations include basement membrane and arteriolar thickening, capillary microaneurysms, and reduced capillary density [165]. A variety of pathological lesions of the small, intramural arterioles, capillaries, and venules have been described in the diabetic heart and ultrastructural studies have demonstrated a significant increase in the thickening of the basement membranes of small blood vessels in the diabetic heart [165]. However, the relative contributions of these lesions to the pathogenesis of diabetic heart disease are still unknown.

Following a study of endometrial biopsy in diabetics with and without hypertension, Harrower and colleagues [166] have suggested that the aforementioned alterations in the microvasculature may cause diabetic cardiomyopathy by causing myocyte injury and interstitial fibrosis. Additionally, microvascular spasm causes myocyte necrosis and resultant areas of fibrosis lead to loss of contractility and reactive hypertrophy, both of which are well recognized features of the diabetic heart [167]. From a functional perspective, the contribution of small vessel disease to cardiomyopathy is summarized succinctly by Fang et al. [165] where they report that "The association of small vessel disease with diabetic cardiomyopathy is supported by the observation that similar abnormalities in coronary small vessel function occur in both diabetes and dilated cardiomyopathy, maximal pharmacological coronary flow reserve is reduced, and endothelium-dependent coronary vasodilation is impaired in both dilated cardiomyopathy and diabetes mellitus." Considering both structural and functional abnormalities of the coronary microcirculation contribute to development of LV dysfunction through episodes of silent MI, the notion that

microcirculatory defects contribute to diabetic cardiomyopathy is attractive.

Coronary microvessel dysfunction might also contribute to CHD and some groups are of the opinion that CHD in the diabetic heart starts early in the course of DM, not only as a disease of the epicardial vessels but also as a small vessel disease [168]. Proposed events in the pathogenesis of microcirculatory dysfunction parallel previously discussed mechanisms involving hyperglycemia, increased oxidative stress, and endothelial dysfunction. Scognamiglio et al. [169] have shown a relation between acute postprandial hyperglycemia and myocardial perfusion defects in type 2 diabetic patients. Following these observations, they suggest a primary role of deterioration in microvascular function in causing myocardial perfusion defects, thereby representing an early marker of the atherogenic process in the coronary circulation. Some studies have documented a reduced coronary flow reserve in diabetics by Doppler echocardiography and others have observed a reduced myocardial perfusion in diabetic patients with angiographically normal coronary arteries using PET [170]. From these analyzes, it might be concluded that the microcirculation becomes affected in DM even before the onset of coronary atherosclerosis. Also, as previously highlighted the dysfunction of the Vv further contributes to atherosclerosis and CAD.

Although convention partitions the vascular complications of DM into the subtypes of macro- and micro-vascular disease a significant body of evidence exists to suggest common pathological interactions. In diabetes of long duration and the metabolic syndrome, small- and large-vessel diseases have been observed to frequently coexist, and have been acknowledged to synergistically contribute to the detriment of the patient. A prominent example is the association between erectile dysfunction and cardiovascular risk that has gained much attention in recent years [171]. Even in the present context, interrelated micro- and macro-vascular pathologies appear to interact to produce detrimental effects, and this statement may be justified by a study of nephropathy in DM where researchers observed that patients with diabetic nephropathy succumb to fatal CAD before the onset of end stage renal failure [172]. In the same vein, the Atherosclerosis Risk in Communities trial demonstrated a correlation between retinopathy and CAD in women. The observation that retinopathy in patients with type 2 DM was concurrent with greater mortality after percutaneous coronary intervention [173] provides further evidence of the interaction of large and small vessel disease in terms of contribution toward the burden of heart failure in diabetes. However, the pathogenesis of microvascular disease in CV risk remains obscure and further investigation in a more focussed fashion is warranted.

Concluding remarks

The prevalence of diabetes mellitus and diabetes-related atherothrombotic macrovascular disease has risen to epidemic proportions especially in the industrialized world. In view of type 2 DM, this trend correlates strongly with increasing obesity and is epitomized by a documented earlier age of onset [88]. Much research is ongoing in the field but the disease still has a stereotyped definition that is based on the subjective detachment of causative factors [163], which renders a certain degree of ambiguity in interpretation of experimental results when the multi-factorial nature of DM is taken into consideration. Thus, while the burden of cardiovascular disease is obvious, its etiology and implications in patients with diabetes are at best, incompletely understood. Various biochemical mechanisms speculated to be at the "heart" of diabetic macroangiopathy have been reviewed in the present article. The leading contenders include the direct and indirect consequences of hyperglycemia and its immediate biochemical sequelae, IR, and dyslipidemia. By labyrinthine interactions, these factors yield a complex (dys)metabolic environment characterized by chronic inflammation, procoagulability, impaired fibrinolysis, neovascularization abnormalities, and microvascular defects that cumulatively alter blood rheology, artery structure, and homeostasis of the endothelium. Manipulation of these initiators of macroangiopathy underlies the current strategy for optimal cardiac care in the diabetic patient. This includes the early detection and aggressive management of traditional CV risk factors to optimize glycemic status, lipid, and blood pressure control [174]. Broadly speaking, the literature is optimistic in that proven therapy and guidelines for lifestyle changes are currently being applied, which significantly improve prognosis and life expectancy in diabetic patients with macrovascular compromise. Nevertheless, considering the magnitude of excess risk of CAD in diabetics, the need to further develop non-standardized therapeutic goals and multi-factorial interventions to address risk factors, and reduce acute coronary events is pressing [74]. Further molecular and functional dissection into the pathobiology of the atherosclerotic vascular wall in DM holds promise in this regard.

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