Molecular Mechanisms Underlying Vascular Disease in Diabetes

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7.1 Introduction

Diabetes mellitus is the heterogeneous derangement of metabolism characterized primarily by chronic hyperglycaemia and insulin resistance [1]. This is due to impaired insulin secretion and/or impaired insulin action [2]. Among all types of diabetes, type 2 diabetes, which was formally referred to as noninsulin-dependent diabetes or adult-onset diabetes, accounts for 90%–95% of all diabetes. Hypertension and type 2 diabetes are common comorbidities that are inextricably linked [3–5]. The former is twice as frequent in patients with diabetes compared with those who do not have diabetes. Patients with hypertension often exhibit insulin resistance and are at greater risk of developing diabetes than normotensive individuals [6]. As comorbidities, hypertension and diabetes correlate with worse outcomes and more

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A. E. Berbari, G. Mancia (eds.), *Blood Pressure Disorders in Diabetes Mellitus*, Updates in Hypertension and Cardiovascular Protection,
https://doi.org/10.1007/978-3-031-13009-0_7

disability than in patients with only diabetes or hypertension [7]. Type 2 diabetes typically occurs in the setting of abdominal obesity, hypertension, hyperlipidaemia and increased coagulability, features that are also common in metabolic syndrome.

Many of the complications of diabetes are linked to vascular injury [8]. Vascular changes typically involve inflammation and prothrombotic processes that manifest as capillary basement membrane thickening, vascular fibrosis, microvascular calcification and endothelial dysfunction [4, 8]. These vascular changes are amplified in obesity and changes in the gut microbiome may be a trigger for metabolic inflammation in obesity and diabetes [9]. Molecular processes underlying these events include oxidative stress, immune responses, activation of the renin-angiotensin system and formation of advanced glycation end products (AGEs) [4, 10]. Recent data indicate an important role for microRNAs in the vasculopathy of diabetes [11]. Hypertension and obesity are important risk factors for diabetes-associated vascular complications, because these conditions are also associated with vascular dysfunction and injury.

This chapter provides a comprehensive update on vascular complications of diabetes and the molecular mechanisms that underlie the vasculopathy of diabetes. In particular, the role of advanced glycation end products (AGEs), oxidative stress and inflammation are highlighted.

7.2 Macrovascular and Microvascular Disease in Diabetes

Diabetes is associated with both macrovascular (large arteries) and microvascular disease (small arteries and capillaries). Macrovascular disease leads to myocardial infarction, stroke and peripheral artery disease, primarily due to atherosclerosis. The process of atherosclerosis is accelerated in diabetes [12–14]. Patients with type 2 diabetes have poorer cardiovascular outcomes than patients without diabetes [15]. Diabetes is a frequent and strong risk factor for large artery disease and coronary artery calcification [16]. Individuals with diabetes consistently have higher levels of calcification than do those without diabetes [16]. Vascular calcification and atherosclerosis in diabetes contribute to increased risk of myocardial infarction. Type 2 diabetes acts as an independent risk factor for the development of ischaemic disease. Major modifiable risk factors for macrovascular disease in diabetes are hypertension, dyslipidaemia, obesity and cigarette smoking [17, 18]. Increased risk of cardiovascular disease starts during prediabetes in association with insulin resistance and impaired glucose tolerance [19].

Microvascular disease leads to retinopathy, nephropathy and neuropathy with target organ damage. These are the major causes of morbidity and mortality in patients with diabetes [20–22]. Microvascular dysfunction seems to precede structural vascular changes. During the early phases of diabetes and/or cardiometabolic disease, each can cause reversible microvascular damage with associated dysfunction. With time these changes may become irreversible leading to target organ damage and consequent vision loss, renal insufficiency and neuropathy [23]. Microvascular disease in diabetes can also cause heart failure, sarcopenia, cognitive decline and worsening of metabolic dysfunction [24]. Processes underlying microvascular injury include increased endothelial permeability, inflammation and oxidative stress [10, 23]. Diabetic retinopathy is

the most common microvascular complication of diabetes often leading to blindness [4]. Diabetic nephropathy, characterized by microalbuminuria, is the leading cause of end-stage renal disease worldwide [25]. Microalbuminuria commonly coexists with hypertension and may reflect endothelial dysfunction in both conditions. Although the underlying cause of microalbuminuria is controversial, it is thought to be a renal manifestation of generalized vascular endothelial dysfunction and is strongly linked to increased cardiovascular risk [26]. Moreover, systemic inflammation precedes microalbuminuria in diabetes, suggesting that by the time microalbuminuria is detected, there is already evidence of vascular injury [26]. Accordingly, screening for microalbuminuria is important for the intervention and prevention of further complications such as end-stage renal disease and cardiovascular disease.

7.3 Pathophysiology of Vascular Disease in Diabetes

7.3.1 Insulin Resistance

Physiologically, insulin maintains glucose homeostasis by integrated actions on carbohydrate, protein and lipid metabolism [27]. These actions occur mainly in the liver, skeletal muscle and adipose tissue. Glucose can alter insulin sensitivity in muscle and fat, as well as decrease insulin secretion from β-cells of the pancreatic tissue. In pathological conditions, hyperglycaemia promotes loss of sensitivity to insulin in insulin-sensitive tissue resulting in insulin resistance, which is associated with type 2 diabetes, obesity, hypertension and other cardiometabolic diseases [28, 29]. Many factors play a role in insulin resistance including AGEs, which inhibit insulin signalling by increasing Ser-307 phosphorylation of IRS-1 and forming methylglyoxal-IRS-1. In addition, in the context of obesity, adipocytes undergo hypertrophy and assume a pro-inflammatory phenotype, which contribute to vascular injury in diabetes [30, 31]. These changes have been shown to coincide with the onset of insulin resistance and provide a pathophysiological link between metabolic and vascular disease.

Activation of the renin-angiotensin system plays an important role in vascular inflammation and injury in diabetes and hypertension [32, 33]. Ang II opposes the actions of insulin to enhance glucose uptake in skeletal muscle and may lead to insulin resistance in the vasculature [34]. Important cross-talk between insulin and Ang II signalling has been demonstrated in VSMCs, where Ang II opposes the effects of insulin [35].

7.3.2 Endothelial Dysfunction

Endothelial dysfunction is a key feature in vascular disease and is typically observed in hypertension, diabetes and obesity [36, 37]. Impaired endothelial function is associated with reduced vasorelaxation, inflammation, prothrombotic state, increased permeability and increased production of vasoactive and mitogenic factors [38, 39]. Abnormal endothelium-dependent vasodilatation may also contribute to or exacerbate insulin resistance by reducing the delivery of glucose to target tissues [40].

7.3.3 Vascular Remodeling

The vasculopathy of diabetes is associated not only with functional alterations, but with structural changes of small and large vessels [41]. Vascular smooth muscle cells (VSMCs) undergo dedifferentiation from a contractile phenotype to a pro-migratory and proliferative form [42]. In addition, they produce pro-inflammatory mediators and pro-fibrotic factors that contribute to chronic low-grade inflammation, vascular fibrosis and increased stiffness, which resemble processes that occur with 'vascular ageing' [43–45]. The vasculopathy of diabetes has been considered as a condition of 'premature' vascular ageing, similar to what has been described in hypertension, since the vascular changes observed in diabetes in young individuals is similar to that observed in non-diabetic elderly people [46].

7.4 Molecular Mechanisms of Vascular Dysfunction and Damage During Diabetes

7.4.1 Advanced Glycation End Products (AGEs) and Activation of the AGE-Receptor AGE (RAGE) System

AGEs are a diverse group of macromolecules formed via the process of non-enzymatic glycation of proteins and lipids [47]. This process is accelerated during hyperglycaemia, oxidative stress, ageing, advanced renal disease and inflammation [48]. AGEs accumulate in the extracellular matrix of vessels and contribute to vascular damage in diabetes [49]. AGEs interact with two main types of cell surface receptors: scavenger receptors, which remove and degrade AGEs, and receptors for AGEs (RAGE), which trigger specific cellular signalling responses on AGE binding [50]. AGEs stimulate the production of reactive oxygen species (ROS), which reversibly enhance AGE formation [51, 52]. AGEs are antigenic and induce immune and inflammatory responses [53]. RAGE is a receptor and member of the immunoglobulin family and binds many ligands besides AGEs. AGE-RAGE signals through transforming growth factor (TGF)-b, NFκB, mitogen-activated protein kinases (MAPK; ERK1/2, p38MAPK) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Nox) and induces expression of vascular adhesion molecule 1, E-selectin, vascular endothelial growth factor and pro-inflammatory cytokines (IL-1b, IL-6, TNF-a) [54].

In diabetes, activation of AGE-RAGE signalling pathways is increased in VSMCs leading to inflammation, pro-thrombotic effects, fibrosis and calcification, which underlie diabetic nephropathy, retinopathy, neuropathy and atherosclerotic cardiovascular disease [55]. In the presence of hypertension these processes are amplified leading to accelerated vasculopathy in diabetes [56]. Patients with diabetes have increased tissue and circulating concentrations of AGEs and soluble RAGE, which predict cardiovascular events [57]. Accordingly urinary and plasma AGE levels and soluble RAGE have been considered as putative biomarkers for vascular disease in diabetes [58] (Fig. 7.1).

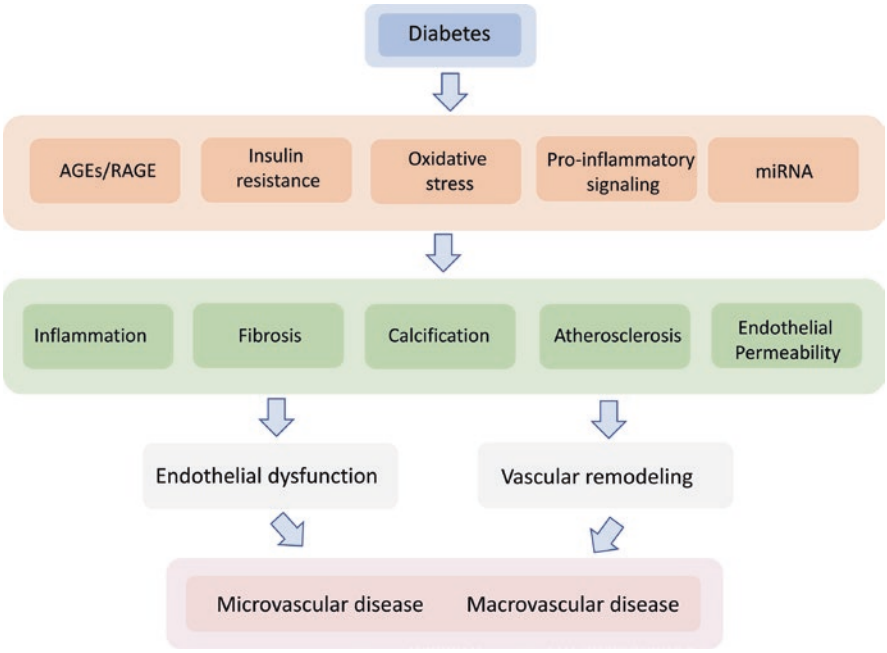

Fig. 7.1 Schematic demonstrating vascular processes whereby diabetes predisposes to microvascular and macrovascular disease, which leads to cardiovascular disease. Activation of AGE/RAGE signaling, oxidative stress, pro-inflammatory signaling and miRNAs lead to vascular injury and dysfunction that manifest as microvascular and macrovascular disease. *AGEs* Advanced glycation end products, *RAGE* Receptor AGE

7.4.2 Oxidative Stress and Vascular Injury in Diabetes

Oxidative stress (increased bioavailability of ROS) is a key mechanism of glucotoxicity in diabetes, as evidenced by increased vascular ROS generation in response to hyperglycaemia and accumulation of oxidation by-products of lipids, proteins and nucleic acids [59, 60]. NADPH oxidases (Nox) and dysfunctional eNOS are principal sources of increased vascular ROS in diabetes [61, 62]. Diabetes-/hypertension-associated oxidative stress is caused by multiple processes that increase and decrease pro-oxidant and antioxidants, respectively [63]. Increased vascular oxidative stress in diabetes and hypertension promotes posttranslational oxidative modification of proteins, causing cellular damage, endothelial dysfunction and vascular inflammation and injury. Oxidative stress and activation of Noxs are increased in patients with diabetes and in preclinical models of diabetes and obesity [62, 64].

Of the seven Nox isoforms (Nox1–5, Duox1, Duox2), Nox1, Nox2, Nox4 and Nox5 have been implicated in cardiovascular and renal oxidative stress in diabetes [65–68]. Nox1 and Nox4 are important in renal injury and atherosclerosis in mouse models of diabetes [66–68]. Nox5 may also be important in diabetes-associated vascular injury and nephropathy [69, 70]. Renal Nox5 expression is increased in

patients with diabetic nephropathy [69]. In transgenic mice with podocyte-specific expression of human Nox5, renal injury was amplified by diabetes [71]. Similar findings were observed in mice expressing human Nox5 in a VSMC-specific manner [72]. Vascular/mesangial cell Nox5 overexpression is associated with amplification of atherosclerosis in mouse models of diabetes [73].

Targeting Noxs has been considered a promising strategy to ameliorate the vasculopathy and nephropathy associated with diabetes. While extensive experimental evidence showed a renoprotective effect of Nox1/4 inhibition in preclinical models of diabetes, clinical studies have been less positive [74, 75]. A clinical trial using GKT137831, a Nox1/4 inhibitor, failed to show improvement in renal function in patients with diabetic nephropathy [76]. Whether targeting Nox5 may have better clinical outcomes is unclear. Ongoing clinical studies are addressing this and the results are awaited.

7.4.3 Hyperglycaemia and Vascular Signalling

In diabetes, hyperglycaemia stimulates mitochondrial respiration and induces endoplasmic reticulum (ER) stress [77]. It also decreases vascular antioxidant capacity, reduces activity of the transcription factor nuclear factor-erythroid 2-related factor (Nrf-2) and promotes activation of vascular Nox isoforms leading to oxidative stress in diabetes [63, 68]. Oxidative stress is also associated with reduced bioavailability of the vasodilator nitric oxide (NO) and increased production of injurious peroxynitrite, causing endothelial dysfunction and inflammation [78]. At the molecular level hyperglycaemia induces activation of redox-sensitive protein kinase C (PKC), MAPKs, calcium channels, pro-inflammatory genes and polyol and hexosamine pathways, further contributing to mitochondrial dysfunction, oxidative stress, ER stress and consequent vascular inflammation and damage [62, 79].

7.4.4 Inflammation and Vascular Injury in Diabetes

It is well established that inflammatory polarization of immune cells occurs in many tissues, including adipose tissue, heart, kidney, skeletal muscle, liver, gut and vessels [80]. Subclinical inflammation contributes to obesity-linked metabolic dysfunctions, leading to insulin resistance and type 2 diabetes mellitus. Obesity triggers metabolically activated immune cells thereby contributing to the adverse regulation of adipocyte metabolism and adipose tissue remodelling [81]. These processes involve activation of many signalling pathways including upregulation of transcription factors such as hypoxia-inducible factor (HIF1α) [82]. Activation of HIF1α induces adipocyte expression of chemokines such as MCP-1, which contributes to adipocyte inflammation through pathways involving the JAK1/JAK2/STAT1 pathway [83]. Circulating and locally produced effector cytokines such as TNF-α, interferon-gamma (IFN-γ), IL-1β and IL-12 [84, 85] may influence the insulin sensitivity of peripheral tissues and, in the pancreatic islets, can modulate insulin

release [86, 87]. Increased glucotoxicity and lipotoxicity have been associated with immune cell infiltration of target tissues, thereby affecting diabetes-associated target organ damage and cardiovascular complications [87–89].

Epigenetics is another mechanism that may influence inflammation and immunometabolism in diabetes [90]. Histone deacetylase (HDAC) inhibitors cause NFκB inhibition through acetylation of the p65 subunit. ITF2357, an orally active HDAC inhibitor, has been shown to prevent the development of diabetes [91]. Similarly, activation of sirtuin1 (Sirt1), involved in inflammation, metabolism and ageing, has been shown to have anti-inflammatory properties in diabetes [92].

Extensive experimental evidence has shown a close association between vascular inflammation, diabetes and cardiovascular morbidity [90, 93]. This is already evident in prediabetes [94]. Clinical studies also support the role of inflammation in cardiovascular complications of diabetes. Patients with type 2 diabetes have increased total leukocyte counts, particularly neutrophils and lymphocytes, that correlate with insulin sensitivity [58], and inflammatory changes of adipose tissue [95–97]. The link between inflammation, insulin resistance and type 2 diabetes is further supported by genetic studies and clinical trials showing the protective effects of immune-targeted therapies and anti-inflammatory actions of classical antidiabetic drugs [98].

To further support the notion that inflammation and activation of the immune system are involved in the pathophysiology of diabetes and its vascular complications, studies integrating metabochip approaches with GWAS have shown that classical immunometabolic genes including JNK signalling pathways, NFκB regulators (MACROD1), inflammasome activators (NRF3) and interferon gamma receptor genes associate with type 2 diabetes [99, 100]. This also corresponds to results of GWAS that identified genes related to macrophage function and antigen presentation. Inflammation and oxidative stress are thus key elements underlying vascular disease and cardiovascular complications in diabetes [101].

7.5 MicroRNAs, Diabetes and Vascular Complications

MicroRNAs (miRNAs) are a group of small, single-stranded, 22–25-nucleotide-long, non-coding RNAs that are multifunctional [102]. They normally bind to the 3′ untranslated region of their target mRNA, leading to translational inhibition and/or mRNA degradation. miRNAs regulate over 90% of all protein-encoding mRNAs and their biological events [103]. They are detected in blood serum/plasma as well as in urine, saliva, tears and breast milk. Over 1000 miRNAs discovered in the human genome have been recognized to be useful diagnostic indicators. They fine-tune gene expression and have been implicated in various pathological processes including diabetes, insulin resistance and cardiovascular disease.

Normally, miRNAs are essential in maintaining physiological homeostasis, metabolism and energy balance. With respect to insulin biology, they control β-cell genesis, β-cell death (miR-21), insulin production (miR-30d, miR-204, and miR-124a) and α/β-cell mass balance (miR-375) [104, 105]. miRNAs are crucial in

regulating adipogenesis (formation of adipocytes), metabolic homeostasis and endocrine functions of adipocytes [106]. Many miRNAs have been identified to be differentially regulated during adipogenesis, including let-7c, miR-143, miR-210, miR-221, miR-27 and miR-30a-e [106, 107]. In obesity, the expression of miR-132 is downregulated and its expression level is related to the activation of NFκB signalling and transcription of MCP-1 and IL-8. Expressions of miR-132 and miR-155 are also associated with macrophage infiltration in adipose tissue [106, 107].

In pathological conditions such as diabetes mellitus and cardiovascular disorders, miRs are differentially expressed [108]. Pancreatic β-cell-specific miRNAs, including miR-375, miR-124a, miR-96, miR-7a, miR7a2, miR-30d, miR-9, miR-200, miR-184 and let-7 are dysregulated in diabetes [109]. Differential miRNA signatures have been identified in prediabetic individuals, diabetic patients and patients with diabetes and vascular complications, suggesting that miRNAs may be novel biomarkers [110]. Diabetic cardiovascular complications are associated with increased levels of miR-223, miR-320, miR-501, miR504 and miR1 and decreased levels of miR-16, miR-133, miR-492 and miR-373 [110, 111]. Detection of deregulated miRNA profile in circulating peripheral blood cells or vascular cells may potentially be associated with diabetes-associated vascular disease.

7.6 Conclusions

Diabetes is associated with an increased risk of cardiovascular disease, which is exaggerated with coexistent hypertension and obesity. Many of the underlying molecular mechanisms, including oxidative stress, inflammation and fibrosis, causing microvascular and macrovascular complications in diabetes, also cause vascular remodelling and dysfunction in hypertension. Preventing vascular injury and inflammation in diabetes may protect against the devastating complications associated with retinopathy, nephropathy and neuropathy. Some of the newer anti-diabetic drugs seem to have vasoprotective effects.

Acknowledgements This work was supported by grants from the British Heart Foundation (BHF) (RG/13/7/30099, RE/13/5/30177).

Conflict of Interest There are no conflicts to declare.

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