

The Prevalence, pathophysiology, molecular mechanisms and biochemical pathways in the pathogenesis diabetic retinopathy– an overview.

Abstract :

Diabetic retinopathy is the leading cause of blindness world over. Neurovascular degeneration of the retina is considered presently the cause as against the vascular changes which are believed to be causative previously. In fact the neuronal changes are shown to ante-date the vascular changes . There is complex interaction of many cells like pericytes, miller cells ,astrocytes , vascular endothelial cells as well as factors like advanced glycation products, Oxidative and metabolic stress , inflammatory cytokines, leukotrienes ,Interleukins , various growth factors like vascular endothelial growth factor (VEGF), neurotrophins etc. and glycoproteins etc. in the pathogenesis of DR. . Various biochemical pathways like Polyol pathway, glucosamine pathway, AGE pathway and PKC pathways interacting with one another is also recognised as having a role in the pathogenesis of DR.. But the common link responsible for all these facto involvement linking to DM2 is still not clear. The mechanism explaining Benfotiamine, a thiamine analogue found to be useful in treating DR , though attractive in integrating the various biochemical pathways cited above, is not comprehensive. The concept of ROS (reactive oxygen spices) a better alternative explanation linking up all pathogenic factors concerned. to chronic hyperglycaemia of D M 2. But antioxidants proved futile in treating DR. It may be useful to remember that extensive ROS production in DM2 is consequent to shift of energy metabolism from glycolysis to B-oxidation of fats.. unless this is reversed , ROS production continues . The Diabetes Control and Complications Trial (DCCT) and UKPDS are the two landmark clinical trials that clearly showed the relationship between chronic hyperglycaemia and genesis of DR. progression in Type 1 DM and Type 2 DM patients, respectively. Randomized controlled trials have shown that early treatment of DM2 can reduce an individual's risk of severe visual loss by 57%.ntensive glycaemic control appeared long lasting because of the metabolic memory, also known as 'legacy effect' , a tern which explains the beneficial effects of immediate intensive treatment of hyperglycaemia with a sustained benefit with respect to the outcomes for many years, regardless of glycaemia in the later course of diabetes. Hence the current emphasis is prevention of DR with strict glycaemic control in DM2 ab initio.

Introduction :

Diabetic retinopathy (D R) is an **unique, chronic, complex, progressive , degenerative disease of the neuro-vascular unit** of Retina.

It is **uniquebecause**

a) It is the leading cause of blindness word-wide

39 b) Approximately 6% of diabetics have already evidence of DR when DM2 is first diagnosed.
 40 c) It is present in 70 % of DM2 pts with duration equal to or greater than 20 years.

41 d) 60% of patients with Type 2 diabetes are expected to have some form of retinopathy by the first
 42 decade of incidence of diabetes [1]

43 e) The changes once started are irreversible though salvage of what is not damaged is possible.

44 It is **chronic** because the disease insidiously develops over a decade or two, until which it remains
 45 asymptomatic . Periodic screening by funduscopy is the only way to detect early retinal changes.
 46 It is **complex** because there is no single identifiable factor that alone links hyperglycemia to DR,
 47 though multiple factors in the pathogenesis are identified. There is no single target identified for
 48 intervention, nor a single factor is attributed to any one particular pathological feature of DR. This
 49 implies that Complex interplay of several factors result in DR.

50 It is **progressive** because, it proceeds from one stage to other relentlessly. To be more precise , DR
 51 proceeds clinically. from less severe through moderately severe to severe stage of NPDR. From this
 52 stage it is computed that in 70 % cases it proceeds to PDR in one year. The ultimate end-point of
 53 both types of DRs is loss of vision.

54 It is a **degenerative** disease because both, neuronal(which includes neurones, photoreceptor cells
 55 and supporting cells) as well as vascular degenerative changes mark the underlying pathology of DR.
 56 **Neurovascular unit degeneration** is favoured than only the vascular changes believed to be
 57 responsible in earlier years, in the pathogenesis of DR. In fact it is now conceded, that neuronal
 58 changes anti-date the vascular changes in DR. However which is the cause and which is
 59 the effect, still remains, a million dollar question.

60 **Global Prevalence :**

61 Globally, there are approximately 93 million people with DR, 17 million with proliferative DR, 21
 62 million with diabetic macular oedema and 28 million with VTDR . [2]

63 et al. also reported racial differences in the prevalence of diabetes but not diabetic retinopathy, with
 64 the highest prevalence seen in the Indian (28.9%) and, followed
 65 by the Malay (24.8%) and Chinese (20.1%). [3] The reported incidence in some of the studies is as
 66 follows - Lian et al. (39%) in Hong Kong, Rodriguez-Poncelas et al. (12.3%) in Spain, , Huang et al.
 67 (33.9%) in Singapore, Giloyan et al. (36.2%) in Armenia, Hajar et al. (27.8%) in Saudi Arabia, and
 68 Dutra Medeiros et al. (16.3%) in Portugal.

69 **Prevalence of DR in India:**

70 As reported by various authors are presented here. The differences in figures may be due to year of
 71 reporting , or other variables of sampling and analysis. Raman et al. (18.1%), Rema et al. (17.6%),
 72 Namperumalsamy et al. (10.6%), Narendran et al. (26.2%) and Dandona et al. (22.58%),
 73 The prevalence in the AIOS (Association of Indian Ophthalmologist Society) study (2014) was 21.27%
 74 with a range of 12.27% in the central zone and 34.06% in the north zone. Thus the AIOS study
 75 brings out the regional differences and range of prevalence.

76 **Magnitude of the problem :**

77 Population of patients suffering from DM 2 are expected to rise to 380 million by 2025 as per one
 78 study, with approximately 4 million people around the world losing their sight from diabetic
 79 According to the World Health Organization, it is estimated that the total number of

people with diabetes will double from 171 million in 2000 to 366 million by 2030..[4]The number of DR and vision-threatening DR (VTDR), which includes severe non-proliferative DR, proliferative DR (PDR)and diabetic macular oedema (DME), has been estimated to rise to 191.0 million and 56.3 million, respectively by 2030.[5] According to the World Health Organization (WHO), is estimated that DR accounts for 4.8% of the number of cases of blindness (37 million) worldwide.[6]

DR as a continuous spectrum of events leading ultimately to loss of vision :

Looking from the pathogenesis point of view, both the NPDR (non proliferative) and PDR(proliferative diabetic retinopathy , appear to pursue two different and independent pathways converging on to a single final outcome ie. blindness. Macular oedema which is the end result of NPDR is preceded by increased capillary perfusion pressure resulting in seepage of fluids and macromolecules from the dysfunctional vascular endothelium(inner retinal barrier) and pigmented neuro-epithelial layer (outer retinal barrier) into retina, limited by its internal limiting membrane (ILM). Whereas, the PDR is preceded by capillary dropout and non-capillary perfusion,leading to hypoxia induced neovascularisation which crosses the ILM and encroaches on to the vitreous and even spreads to iris to involve the trabecular opening causing glaucoma. Even the pathogenic factors operating behind the both conditions seems to be different ie. cytokines and leukotrienes are implicated in the vascular changes of NPDR whereas growth factors like VEGF etc. are implicated in the genesis of PDR. The other factors like AGEs, oxidative and metabolic stress etc have been implicated in changes leading to both the types. But there is considerable overlap in the spectrum is supported by the facts that the macular oedema is not the exclusive feature of the initial NPDR but can occur at any stage. When it occurs at a late stage the newly formed vessels themselves are responsible for it. The ischemia of capillaries begins in the NPDR stage itself, especially in the severe type of NPDR. This transitory stage between the NPDR and PDR is known as **Diabetic Macular ischemia**-(DMI). Hypoxic ischemia is common to both DMI and PDR and the presence of neovascularisation distinguishes the later. The **micro-aneurysms**, the earliest change in DR are seen in both the types, This earliest stage of DR is called **Background retinopathy**. But the **micro aneurysms of PDR** are formed from **neovascularisation**, and cross the ILM a differentiating point between DMI and PDR. Further, PDR is never seen de novo and is always preceded by NPDR. The conceptualisation of both entities as a part of continuous spectrum is advantageous in the sense that detection of earlier (NPDR) changes call for stringent management of hyperglycaemia to keep at bay the late and more serious sequelae of PDR. To this extent the spectrum of events can be viewed as progressing in orderly fashion, characterised by appearance of microaneurysms which bleed to produce succeeding events consisting of **haemorrhages** and **cotton wool exudates**. The foveal thickness decreases as evidenced by OCT (optical coherence tomography) and avascular zone size increases as seen in FA (Fluorescent angiography). **macular oedema is seen earlier in NPDR** , the severe form of the latter is called **DMI** which is a transitional stage between the two types of DR . From here the progression is to capillary nonperfusion-hypoxia -**neovascularisation** leading to **PDR** .

Diabetic Macular Ischemia (DMI)

DMI is defined by an enlargement of the foveal avascular zone (FAZ) and paramacular areas of capillary nonperfusion. Large proportion (approximately 41%) of patients with DR in a tertiary hospital setting have some evidence of macular ischemia. Capillary nonperfusion, capillary drop out and arteriolar narrowing are behind development of DMI. The events that lead to DMI are -

- **Endothelial proliferation,**

- 125 • Platelet and leukocyte adhesion to endothelial wall,
- 126 • Occlusion and thrombosis of vessel
- 127 and compromised microcirculation and consequent anoxia.

128 **Role of hyperglycaemia in D R**

129 The **Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes**
 130 **Study (UKPDS)** clinical trials confirmed the strong relationship between chronic hyperglycaemia and
 131 the development and progression of diabetic retinopathy. Randomized controlled trials have shown
 132 that early treatment can reduce an individual's risk of severe visual loss by 57%.(7).

133 The crucial role played by hyperglycaemia gradually unfolds as we proceed, as it is the initiator of
 134 diverse pathological events that culminate in producing DR.

135 **The patho-physiology and molecular and biochemical basis of DR**

136 **Neuro- vascular changes in DR**

137 Diabetic retinopathy is a disease involving neuro-vascular unit, which comprises of retinal
 138 vasculature comprising of capillary endothelium, its supporting structures like pericytes and
 139 astrocytes and inner limiting membrane and neuronal epithelium consisting of various types of
 140 neurons, microglial cells, photoreceptors and outer pigmented layer of retina. Though the
 141 vasculature changes are thought to be important, neuronal changes are shown to precede them in
 142 diabetic retinopathy development. Their cause and effect relationship is debatable.

143 **Neuronal dysfunction in DR:**

144 neuro retinal function is compromised before the onset of vascular lesions in diabetic humans , [8]
 145 The retina is a transparent layer of neural tissue between the retinal pigmented epithelium and the
 146 vitreous body. The retina includes five major cell types that perform sensory, regulatory, nutritional,
 147 and immunomodulatory functions. The neurons (photoreceptors, bipolar, horizontal, amacrine, and
 148 ganglion cells) perform sensory functions and define colour perception, spatial resolution, and
 149 contrast discrimination. The neuroglial cells sub serve supportive and nutritional purpose. Normal
 150 vision depends on intact cell-cell communication among the neuronal, glial, microglial, vascular, and
 151 pigmented epithelial cells of the retina. Electroretinogram studies suggest that the dim light retinal
 152 rod pathway is especially susceptible to diabetic damage.
 153 The macula and peripheral retina operate reciprocally; therefore, the macula is more sensitive than
 154 the periphery in bright light, and the converse occurs in dim light

155 **Role of astrocytes in DR :**

- 156 • Astrocytes are star shaped glial cell present in retina in addition to brain and spinal cord .
- 157 • Glial cells are the interface between the neurons and the vasculature and are thus key
- 158 regulators of neuronal nutrition and metabolism.
- 159 • Vascular leakage occurred with astrocyte loss in early diabetic mice retina as Ang2
- 160 increased. The astrocyte loss and vascular leakage were inhibited by intra - vitreal injection
- 161 of Ang2-neutralizing antibody. In vitro, Ang2 aggravated high glucose-induced astrocyte
- 162 apoptosis via GSK-3 β activation. Ang2 directly bound to α v β 5 integrin, which was abundant
- 163 in astrocyte, and the blockade of α v β 5 integrin, in vitro, effectively attenuated Ang2-
- 164 induced astrocyte apoptosis. In vivo, intra- vitreal injection of anti- α v β 5-integrin antibody
- 165 inhibited astrocyte loss in early diabetic retinopathy. Taken together, these findings suggest

that Ang2 induced astrocyte apoptosis under high glucose via $\alpha v\beta 5$ -integrin/GSK-3 β / β -catenin pathway.

- The perivascular end feet of astrocyte contribute to the BRB (blood retinal barrier) dysfunction features. Astroglial-endothelial signalling regulates the BRB under pathological conditions.
- In addition, astrocyte is closely related to the formation of tight junction in developing retina.[9]
- Retinal astrocytes help to enhance barrier function by releasing soluble proteins. Retinal astrocytes promote the integrity of blood barrier by increasing tight junction protein expression in retinal endothelial cells.[10]
- kinase anchor protein 12 (AKAP12) is derived by astrocytes. AKAP reduces VEGF level and increases TSP-1 levels leading to enhanced blood barrier through the inhibition of PKC- ζ phosphorylation and Rho kinase activity. [11]

Role of mullercells

- Muller's cells are neuroglial cells that extend the span of retina
- Müller cells contribute to neuronal dysfunction,
- The production of pro-angiogenic factors like VEGF leading to neovascularization.
- Set up of a chronic inflammatory retinal environment, and eventual cell death.
- Müller cells produce factors capable of modulating blood flow, vascular permeability, and cell survival,.
- Müller cells recycle neurotransmitters, prevent glutamate toxicity, redistribute ions by spatial buffering, participate in the retinoid cycle, and regulate nutrient supplies by multiple mechanisms.
- Muller glia also contribute to impaired clearance and retinal oedema during diabetic retinopathy.

Portillo et al.'s study suggests that CD40 activation induces Müller cells to release ATP, leading to activation of P2X7 purinergic receptors on retinal microglia and their subsequent expression of inflammatory cytokines. Müller cell-specific CD40 expression is related to the appearance of acellular capillaries in diabetic retina.

Role of pericytes:

Pericytes are contractile cells that wrap around the endothelial cells that line the capillaries and venules . Their role in DR is summarised below.

- **Micro aneurysmformation:**
Pericyte loss is related to microaneurysm formation .[12]It is possibly due to local weakening and subsequent out pouching of the weakened vessel walls. Vessels with micro aneurysms tend not to contain pericytes, suggesting that their loss permits uncontrolled proliferation of endothelial cells, contributing to micro- aneurysm formation in retinal vessels. '*pericyte dropout*' has been used as an index of diabetic retinopathy.
- **Pericyte ghosts formation:**

The selective loss of pericytes from the capillary wall is a histopathological hallmark of diabetic retinopathy, and dropout of these cells is evidenced by the presence of so-called “pericyte ghosts” on histological specimens.

- **Regulation of retinal vascular blood flow :**

Given the potential contractile properties of these cells, it is conceivable that their loss could alter the way blood flow is regulated in the retinal microvasculature. Recently, It is demonstrated that **angiopoietin 2 (Ang2)** induces pericyte apoptosis via **integrin signalling** in DR.¹⁶ .Hyperglycemia-induced Ang2 has a central role in pericyte apoptosis by Ang2/ $\alpha 3\beta 1$ -integrin/p53 signalling pathway in streptozotocin (STZ)-induced diabetic mice. In addition, Ang2 is upregulated by hyperglycemia .

- **Formation . of acellular capillaries, and thickening of the vascular basement membrane**^[13]

Consequently, pericyte dysfunction leads to capillary dilation, micro- aneurysms and increased vascular permeability resulting in vascular leakage and macular oedema progressive vascular occlusions in the human diabetic eye

- **Role in PDR:**

In vitro, pericytes control endothelial cell proliferation , suggesting that pericyte loss may be involved in the pathogenesis of proliferative diabetic retinopathy. Loss and degeneration of pericytes affect the integrity and maintenance of BRB..

- **Role in NPDR :** However, pericyte Loss also occurs in diabetic rodent models, in which proliferative retinopathy is absent. This suggests that pericytes may also control endothelial function during the initial period of non-proliferative diabetic retinopathy.. ^[14]

Cause of pericyte loss in DR

- Hyperglycaemia and local hypertension are known to be a direct cause of pericyte apoptosis dropout, is hypothesised due to toxicity due to accumulation of Sorbitol or AGEs.^[15]

Role of Vascular endothelial cells :

- They actively regulate the vascular tone and permeability, the balance between coagulation and fibrinolysis, the composition of the sub endothelial matrix, the extravasation of leukocytes, and the proliferation of vascular smooth muscle To carry out these functions, the endothelium produces a variety of **regulatory mediators, such as nitric oxide, prostanooids, endothelin, angiotensin II, tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWF), adhesion molecules and cytokines**. Transport of metabolites, fluids , ions and nutrients between blood and the retina is selectively regulated by the physical barrier formed by endothelial cells to maintain homeostasis in the retina.
- Endothelial cells express cell-type specific transmembrane proteins for adhesion including vascular endothelial cadherin (**VE-cadherin**) at **adherens junctions** and **claudins** at tight junctions. ^[16,17]
- Transmembrane proteins at tight junctions include **occludin, claudins** and **junctional adhesion molecules** on .^[18] **Occludin** associates with **zonula occludens (ZO)-1, ZO-2 and ZO-3**, which directly bind to filamentous actin (**F-actin**) .
- Tight junctions act as a gate for Para cellular transport, and a fence for maintaining cell polarity. ^[20]

- At adherens junctions, VE-cadherin is a main component associating with intracellular proteins including **β -catenin, p120, and plakoglobin**. Regulation of VE-cadherin expression and/or phosphorylation can affect the overall endothelial cell junctions and vascular stability through modulation of intracellular signalling pathways.[21]
- Hyperglycaemia effects endothelial cell mitochondria leading to increased ROS level and membrane potential was correlated with the rate of cell death. ([22]104) Hyperglycaemia causes mitochondrial fragmentation with concomitant increases in cytochrome c release leading to apoptosis.[23]
- Hyperglycaemic conditions increase PP2A activity through up-regulation of methylation followed by the activation of apoptotic proteins, superoxide, and NF- κ B. [24]
- apoptosis of retinal endothelial cells under high glucose is independent of caspase activity.[25]
- Hyperglycemia reduces the expression of connexin 43 and decreases cell-cell communication triggering apoptosis.[26]
- high glucose conditions disrupted tight junctions by decreasing occludin expression through the activation of VEGF and insulin-like growth factor-I receptors.[27]
- Hyperglycaemic conditions also affect tight junctions by decreasing ZO-1 levels in the retina of diabetic mice.[28] Consequently, such conditions disturb junctional integrity leading to increased retinal endothelial cell permeability.[29]
- High glucose conditions enhanced the migration of retinal endothelial cells which is mediated through activation of Src, PI3K/Akt1/eNOS and ERKs pathways.[30] as well as through the up-regulation of heparanase. The activation of Akt and ERK phosphorylation are also involved in the up-regulation of heparanase.[31]
- Increased migration under high glucose conditions contributes to increased angiogenic activity of retinal endothelial cells and is consistent with down-regulation of TSP-1 in microvascular endothelial cells under hyperglycemia and enhanced migration of TSP-1-deficient retinal endothelial cells.[32]

Blood retinal barrier (BRB) dysfunction.:

RBB consists of Outer and inner BRB and tight junctions.:

The inner blood-retinal barrier, which is formed by the intra-retinal microvasculature cells surrounded by pericytes and the foot processes of astrocytes.[3] Astro- and pericytes are crucial for maintaining the structure and nutrition of inner BRB.

Both the inner and outer RBB are characterised by tight junctions between adjacent cells which effectively limit the passage of solutes, proteins and fluids from the choroidal and intra-retinal blood supplies

Blood constituents may leak into the retina, resulting in **diabetic macular oedema**, the commonest cause of blindness in diabetes. This is the stage of NPDR. When NPDR is severe, ischemic changes set in leading to a pre-PDR, called **diabetic macular atrophy which progresses to PDR**. The latter is due to anoxia induced neovascularisation.

Outer RBB: Is formed by the pigmented layer of retina. Outer RBB dysfunction leads to increased permeability and leak from chorio-capillaries contributing to macular oedema.

Pathology of DR – a bird's eye view.

Haemodynamic changes

1) Non-proliferative DR.

- 2) narrowing of the retinal arteries and reduced blood flow initially, in short duration of diabetes (less than 5 years)
- 3) Dilation of the retinal arteries and increased retinal blood flow at a later stage .
- 4) retinal perfusion is inversely correlated with levels of glycated haemoglobin in both Type 1 and Type 2 diabetic patients.
- 5) Increased capillary pressure and leakage of macromolecules and fluids
- 6) Macular oedema

2) PDR.

1) Capillary nonperfusion and capillary drop out.

2) Hypoxia induced neovascularisation.

Histo-pathological changes in D R :

❖ **Basement membrane thickening -**

It is a result of the up regulation of membrane components e.g., **fibronectin, collagen IV and laminin**) and/or **reduced breakdown by proteolytic enzymes**[15].

❖ **"ghost pericytes"-**

loss of pericytes- (pockets within the basement membrane that appear to have once contained a pericyte) dysfunction leads to capillary dilation, micro aneurysms and increased vascular permeability resulting in vascular leakage and macular enema.[12]

❖ **Acellular capillaries:**

acellular capillaries are those in which basement membrane or remnant endothelial cell cytoplasm remains while the cell nucleus is absent.¹

❖ **Capillary micro-thrombi, occlusion and capillary drop out^{C)}**

❖ **Arteriolar degeneration.**

❖ **Destruction of vascular musculature**

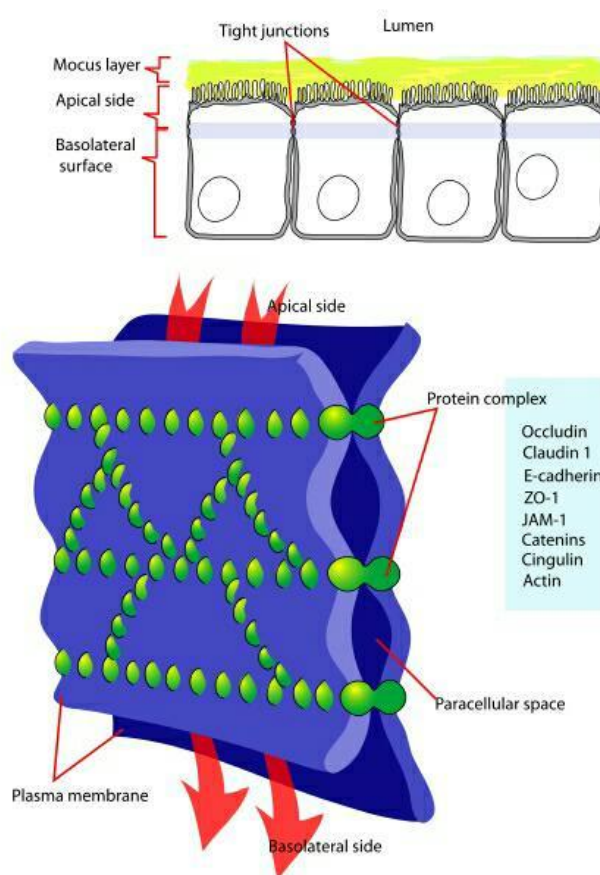
❖ **micro aneurysm formation.**

Tight junction disassembly (Fig 1)

Tight junctions, also known as occluding junctions or zonulae occludens are multiprotein junctional complex whose general function is to prevent leakage of transported solutes and water and seals the Para cellular pathway. Tight junctions may also serve as leaky pathways by forming selective channels for small cations, anions, or water. interaction with PAR3 and PAR6, PKC ζ also phosphorylates occludin and thereby promotes tight-junction assembly, identifying PKC ζ as a key player in the regulation of tight-junction function integrity. [33]TNF- α signals, through PKC ζ /NF-k B pathway alter tight junction complexes and increase retinal endothelial cell permeability. [34] Titchenell et al. showed that PKC ζ inhibitor prevent vascular endothelial growth factor (VEGF) induced blood retinal barrier dysfunction. [35] Similar findings were recently reported in the brain where PKC ζ activity mediated hypoxia-induced increase in cortical blood-brain barrier permeability. [36]PKC ζ is probably activated by ceramide pathway.

Fig 1. Tight junction

Source- author - Mariana Ruiz LadyofHats, Wikipedia.



337

338 Angiopoietins :

339 is part of a family of vascular growth factors that play a role in angiogenesis. angiogenesis, is the
 340 process by which new arteries and veins form from pre-existing blood vessels. Angiogenesis
 341 proceeds through sprouting, endothelial cell migration, proliferation, and vessel destabilization and
 342 stabilization. They are responsible for assembling and disassembling the endothelial lining of blood
 343 vessels.[37]Angiopoietin cytokines are involved with controlling microvascular permeability,
 344 vasodilation, and vasoconstriction by signalling smooth muscle cells surrounding
 345 vessels[38]identified growth factors VEGF with addition contributions of other factors like
 346 **angiopoietin-1, integrins, and chemokines** play an essential role. VEGF and ang-1 are involved
 347 endothelial tube formation. There are four angiopoietins: **ANGPT1, AN**
 348 **GPT2, ANGPTL3, ANGPT4.(4)[39]**

349 Vascular permeability signalling

350 Angiopoietin-1 and angiopoietin-2 are modulators of endothelial permeability and barrier function.
 351 Endothelial cells secrete angiopoietin-2 for autocrine signalling while parenchymal cells of the

extravascular tissue secrete angiopoietin-2 onto endothelial cells for paracrine signalling, which then binds to the extracellular matrix and is stored within the endothelial cells.(7)[40]
 Angiopoietin-1 is critical for vessel maturation, adhesion, migration, and survival. Angiopoietin-2, on the other hand, promotes cell death and disrupts vascularization. Yet, when it is in conjunction with vascular endothelial growth factors, or VEGF, it can promote neo-.vascularization.
 Akt/FKHR pathway in the vasculature is the basis for actions of Ang-1 as a regulator of endothelial cell survival and blood vessel stability.FKHR, regulates many genes associated with vascular destabilization and remodelling (including angiopoietin-2, an Ang-1 antagonist) and endothelial cell apoptosis (e.g., **survivin**, **TRAIL**). Ang-1 inhibits FKHR-mediated changes in gene expression and FKHR-induced apoptosis.
 Ang-1 inhibits, via the **FKHR pathway**, the expression of its antagonist Ang-2 . This regulation implies Angiopoietin-2 is produced and stored in Weibel-Palade bodies in endothelial cells and acts as a TEK tyrosine kinase antagonist. As a result, the promotion of endothelial activation, destabilization, and inflammation are promoted. Its role during angiogenesis depends on the presence of Vegf-a.(9) [41]
 Angiopoietin-2 is produced and stored in Weibel-Palade bodies in endothelial cells and acts as a TEK tyrosine kinase antagonist.

-Relationship of vascular and neurodegenerative changes in the genesis of DR:

Feed forward hypothesis.

This hypothesis tries to explain the interdependence of both neuronal and vascular changes.

feed-forward concept of diabetic retinopathy. (A)Under normal conditions blood vessels supply neural tissue with nutrients. In turn, neural cells produce factors that induce the blood-retinal barrier so the retina can control the local biochemical environment and protect the retina from circulating antibodies, inflammatory cells, and amino acids. (B) In diabetes normal probarrier stimuli from the neural retina may be compromised, allowing blood-borne elements to enter the retina and damage neural cells through a chronic inflammatory process. Neurodegeneration leads to induction of growth factors (e.g., VEGF) and loss of probarrier factors, aggravating vascular damage, including capillary occlusion and permeability. Thus, a feed-forward cycle of combined vascular and neural damage ultimately leads to impaired vision..

Role of Apoptosis:

Diabetes-induced cell death has been observed in numerous retinal cell types such as endothelial cells pericytes , neural retinal cells such as ganglion cells [12-14], and retinal glial cells such as Müller cells, astrocytes, and microglia .

A study showed that the total number of cell bodies in the retinal ganglion cell layer was reduced by 10% after 7.5 months of diabetes. This was accompanied by a 22% reduction in the thickness of the inner plexiform layer and a 14% loss in the thickness of the inner nuclear layer. In contrast, there was no change in the thickness of the outer nuclear layer, suggesting that more cells were lost from the inner than from the outer retina.

Effects of Vascular apoptosis :

acellular capillaries: These are capillaries in which basement membrane or remnant endothelial cell cytoplasm remains while the cell nucleus is absent. 1
 capillary nonperfusion and retinal ischemia whether is the cause or effect of apoptosis is not known.

Effect of Neural apoptosis :

Pericyte ghosts; These are due to neuronal apoptosis

Suggested causes of apoptosis:

- **glutamate toxicity.**
- **Loss of trophic support/ signalling.**
- **Oxidative stress and neuro-inflammation**

Types and pathways of apoptosis;

According to Nomenclature Committee on Cell Death (NCCD) there are now 13 subroutines of regulated cell death identified.[42] These include anoikis, autophagic cell death, caspase-dependent intrinsic apoptosis, caspase-independent intrinsic apoptosis, cornification, entosis, extrinsic apoptosis by death receptors, extrinsic apoptosis by dependence receptors, mitotic catastrophe, regulated necrosis, netosis, parthanatosis, and pyroptosis

‘Extrinsic Apoptosis’ is cell death induced by binding of lethal ligands including FAS/CD95 ligand, tumor necrosis factor α (TNF α), or TNF-related apoptosis inducing ligand (TRAIL) to their respective death receptor.[43] Upon binding of these ligands to the death receptor, the **“death domain”** of the receptor recruits the assembly of the **“death-inducing signalling complex”** (DISC), a platform of various proteins. The DISC results in activation of caspase-8 (32) [44] active caspase-8 initiates one of two distinct pathways. First, active caspase-8 can directly cleave caspase-3, known as an executioner caspase in the apoptotic process (35)[45] Alternatively, active caspase-8 can cleave BH3-interacting domain death agonist (BID) creating truncated BID (tBID). tBID then binds Bcl-2 allowing BAX to form a pore in the outer membrane of the mitochondria enabling the release of cytochrome c into the cytosol. This triggers formation of the canonical **‘apoptosome’** via assembly of **APAF1** with **pro-caspase-9, cytochrome c, and dATP** leading to **caspase-9** activation, which in turn activates caspase-3 (36)[46]

Role of inflammation in DR:

➤ **innate immunity in DR:**

Diabetes-induced inflammation predominantly appears to involve the innate immune system. Experimental studies have shown that high glucose can induce 1) monocyte activation through up regulation of cell surface toll-like receptors (30) AGEs can cause neutrophil activation and dysfunction via the RAGE pathway. 3) monocytes and macrophages express a variety of scavenger receptors, and oxidized LDL may affect monocyte activation through these receptors. Neutrophil, monocyte and platelet activation has been detected in diabetic patients, which is believed to be responsible for the abnormal leukocyte-endothelial interaction (leukostasis) observed in both patients and animal models of diabetes

Leukostasis

- is a sign of microvascular chronic inflammation in the early stages of diabetes, and has been postulated to be a factor of retinal endothelial cell death in diabetes

Leukostasis responsible for :

- **capillary occlusion and capillary non-perfusion** seen in diabetic retinopathy.
- **breakdown of the blood-retinal barrier and increased**
- **permeability of RBM.**
- **degenerative vascular pathology in diabetic retinopathy**

▪ **micro aneurysmsformation**

- The invading pathogens are recognized by pattern recognition receptors, such as Toll-like receptors (TLRs) and the receptor of advanced glycation end products ([36][48]The molecules of the pathogens that bind to these receptors are called pathogen-associated molecular patterns (PAMPs. [49]
- 3) TLR activation or, in absence of pathogens, tissue stress alone, can lead to de-inhibition of the transcription factor Nuclear factor kappa B (NF- κ B), which translocates into the nucleus to stimulate transcription of pro-inflammatory cytokines, acute phase proteins and chemokines, such as IL-6, TNF- α , IL-1 β , and monocyte chemoattractant protein-1 (MCP-1). 3) Inflammation normally resolves promptly through a coordinated program involving **resolvins**, **lipoxins** and **protectins**. [50] When it is not resolved dramatic consequences ensue

➤ **Extravascularinflammation::Role of microglia and mullers cells :**

Microglia and Muller cell activation have been observed in diabetic retina in rats and mice in the absence of clinical pathologies. [51]The activation of microglia in the diabetic retina can lead to the increased production of proinflammatory cytokines, ROS, growth factors, matrix metalloproteinases (MMPs), and nitric oxide all of which have been implicated in the pathogenesis of diabetic retinopathy.

cytokines :

- **Inflammatory cytokines**—IL-1 β , IL-6, IL-8, TNF- α and MCP-1—have been reported elevated in ocular tissues from non-proliferative DR (NPDR) patients. One study detected even higher levels of IL-8 and TNF- α in diabetic eyes with NPDR than with active PDR The increase in these cytokines produced by activated microglia, endothelial cells, macroglia, and later even neurons, highlight the increased activity of these inflammatory cytokines in the early stages of DR and the progression of the inflammatory response throughout all cell types of the retina [52]. The accumulation of these inflammatory mediators has been proposed to contribute to early neuronal cell death in the retina in DM. Some of the cytokines identified, such as MIP-1, IL-1 and IL-3, are thought to also have a role in angiogenesis, as established in experimental ischemic mouse models suggesting that inflammation also contributes and precedes the development of neovascularization in PDR].Such as IL-8, CCL2, have been detected in the vitreous from patients with proliferative retinopathy Increased ICAM-1 and LFA-1 expression was observed in human epiretinal membranes removed from PDR patients. Furthermore, CD11a/CD11c+ or HLA-DR+ monocytes and CD3+ T cells were also detected in epiretinal membranes obtained from PDR patients.
- **Neurotrophins** : Significantly higher vitreous levels of neurotrophins (NTs), a group of specific growth factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3, NT-4, ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF) could be detected in DR patients, with even higher levels in NPDR compared to PDR [51]. This might be an attempt by glial cells such as MGCs to rescue compromised neurons during this early stage of DR.
- **Growth factors**: In patients with PDR, increased vitreous concentrations of the aforementioned cytokines and neurotrophins along with other growth factors such as VEGF, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) have been reported [55].
- **Soluble cytokine receptors**:
Of note, analysis of vitreous samples from PDR patients also revealed increased levels of soluble cytokine receptors, such as sIL-2R [56]. This increase constitutes a known negative

regulatory mechanism of cytokine signaling, suggesting that counter-regulatory mechanisms of angiogenesis and inflammation exist within the eye. note, analysis of vitreous samples from PDR patients also revealed increased levels of soluble cytokine receptors, such as sIL-2R [56]. This increase constitutes a known negative regulatory mechanism of cytokine signalling, suggesting that counter-regulatory mechanisms of angiogenesis and inflammation exist within the eye

Matrix metalloproteinases:(MMPs)

The development of PDR is a multistage event, including angiogenesis, in which basement membrane degradation, endothelial cell migration and proliferation, followed by capillary tube formation, occur. Matrix metalloproteinases (MMPs), which are important regulators of those migratory and tissue remodelling events, have been reported to be upregulated in PDR as well [57].

Role of complement regulators - expression or impaired function of including **CD55, CD59, and DAF**, has been observed in diabetes as a result of nonenzymatic glycation

- **oxidised LDL** related to *Pericyte cell death* is known to initiate inflammatory responses and complement activation. [52]

➤ **iNOS:**

iNOS is an enzyme that catalyses the reaction for nitric oxide (NO) generation. iNOS is up-regulated in the diabetic retina and plays a crucial role in the pathogenesis of vascular lesion during the early stage of DR.[53]

➤ **Leukotrienes :**

by lipoxygenases (LOs), especially 5LO, by leukocytes promotes the retinal capillary degeneration in diabetic mice[34] and contributes to increased levels of pro-inflammatory cytokines. Tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) are increased in the retinas of diabetic animals.[54]

➤ **VEGF:**

Vascular endothelial growth factor (VEGF) also has a pro-inflammatory role and is elevated in the diabetic retina. The increase in VEGF level promotes neovascularization and damage in the retina.[[55]and its inhibition ameliorates vascular leakage in the diabetic retina.(38) [56]

➤ **PEDF:**

Pigment epithelium-derived factor (PEDF) is a member of the super family of serine protease inhibitors. It has neuroprotective, antiangiogenic, and antiinflammatory features.(39) [57] PEDF levels are attenuated in the diabetic retina and associated with up-regulation of VEGF levels. [58]

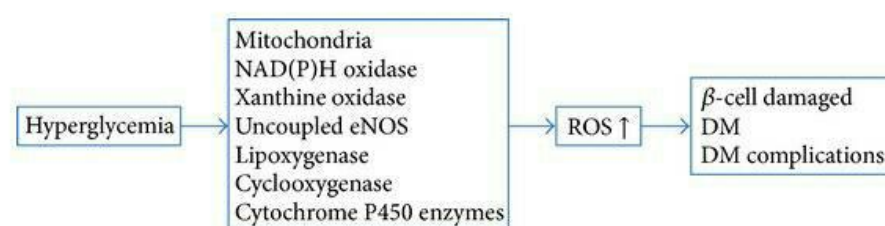
➤ **TSP 1 :**

lack of TSP-1 and/or its selective down-regulation in the endothelium exacerbates the development and pathogenesis of DR. [59]

➤

ROS / Oxidative stress and DRP.((Fig 2)

Fig 2 Mechanisms of ROS production.



527 :

528 -----

529 Diabetes causes oxidative stress, and augmented level of oxidative stress
 530 contributes to the pathogenesis of diabetic vascular complications,
 531 including DR.(20) [60] Reactive oxygen species (ROS) is generated from
 532 1) mitochondrial electron transport chain,
 533 2) cytochrome p450s,
 534 3) the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase
 535 4) nitric oxide synthases (NOSs). ROS produced by cells is required to
 536 maintain normal cellular functions. However, excess production of ROS
 537 result in pathological conditions and oxidative stress

- 538 • Fig 2 Mechanism of ROS production
 539 In the diabetic retina, ROS levels are elevated and related with
 540 vascular dysfunction, including
 541 • loss of pericytes,
 542 • formation of acellular capillaries,
 543 • vascular leakage
 544 • and thickening of BM.(21) [61]

545 Oxidative stress in the diabetic retina has causal links with
 546 vascular inflammation,

547 **metabolic dysfunction due to ROS :**

- 548 1) Vascular endothelial dysfunction.
 549 2) RAGE activation, activation of PKCs,
 550 3) and activation of nuclear factor kappaB (NF- κ B). [62, 63, 64, 65], (23, 24, 25, 26)
 551 Photoreceptor cells have been recently shown to be an early source of oxidative stress and local
 552 inflammation in the diabetic retina.
 553 Diabetes increased generation of ROS by photoreceptor cells followed by induction of pro-
 554 inflammatory mediators, including inducible nitric oxide synthase (iNOS) and intercellular adhesion
 555 molecule 1 (ICAM-1) [66]

556 **Role of Carbonic Anhydrase (CA) IN DR**

- 557 • 1) Gao et al. it was shown that diabetic patients had significantly higher concentrations of CA
 558 than healthy controls . [67]
 559 2) It has been demonstrated that CA inhibitors including acetazolamide and benzolamide
 560 can reduce the progression of diabetic retinopathy and prevent visual loss in both animal
 561 and clinical studies (150) [68]
 562 • **The mechanisms suggested are** 1) reducing humor secretion, inducing vasodilatation .
 563 • 2) improving blood flow to the ocular region,
 564 • 3) inhibiting platelet aggregation
 565 • 4) And reducing vascular permeability.

566 **RASS VS DR**

567

- 1) The expression of the receptors and signalling molecules of RAAS, namely, renin, angiotensin converting enzymes I and II (ACEI and ACE II), as well as angiotensin receptors, has been reported to increase in the retina, during PDR (72, 73) [69,70]
- 2) Studies on experimental models provide evidence that ACE inhibition prevents neovascularisation, a hallmark feature of early diabetic retinopathy (74)][71]
- 3) RAAS contributes to diabetic retinopathy through probably angiotensin II involvement in the PKC activation as well as VEGF signalling.

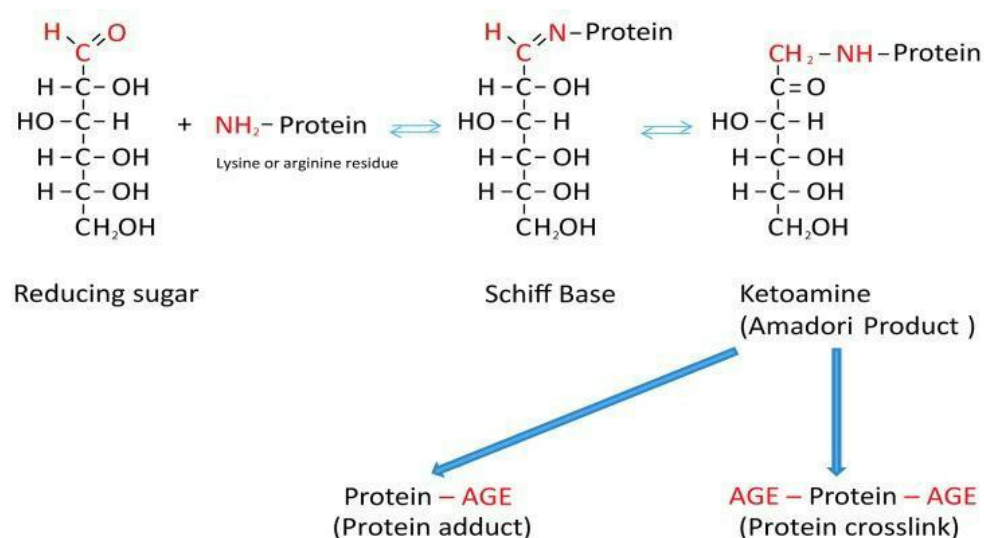


Fig 3 Maillard or browning pathway.

Role of AGEs in D R

Advanced glycation end products (AGEs) are proteins or lipids that become glycated as a result of exposure to sugars. They are believed to play a causative role in the vascular complications of diabetes mellitus. The interaction of AGEs with specific cell surface receptors has been implicated in the development of diabetic retinopathy. These AGE receptors include the RAGE, galectin-3, CD36, and the macrophage scavenger receptor. AGEs affect cells by three main mechanisms: (1) as adducts occurring on modified serum proteins, (2) as endogenous adducts formed as a consequence of glucose metabolism, or (3) as extracellular matrix-immobilised modifications of long-lived structural proteins.

Production of AGEs :

There are two pathways involved in production of AGEs:

1)The Maillard, or browning reaction (Fig3)

It begins with the formation of a Schiff base between glucose and ε-amino groups that slowly rearranges to relatively stable Amadori adducts, an example of which is haemoglobin A1c (HbA_{1c}). Both the Schiff base and the Amadori product can undergo further oxidation and dehydration with concentrations ultimately dependent on both

forward and reverse reactions. The forward reactions give rise to additional irreversible protein-bound compounds collectively termed advanced glycation end products (AGEs). During diabetes, the rate of formation of AGEs exceeds that predicted by first-order kinetics. Thus, over time, even modest hyperglycaemic excursions can result in significant adduct accumulation on long-lived macromolecules.

2) The oxidative pathway:

AGEs are produced from DHAP/GD3PH through dioxy glycol pathway .

AGEs have a range of pathological effects, such as:

- **increased vascular permeability.**
- **increased arterial stiffness Inhibition of vascular dilation by interfering with nitric oxide.**
- **Oxidizing LDL**
- **Binding cells including macrophage, endothelial, and mesangial—to induce the secretion of a variety of cytokines**
- **Enhanced oxidative stress**
- Accumulation of AGE might contribute to increased retinal endothelial cell permeability resulting in vascular leakage [73]. Vessel wall thickening and coagulation, leading to occlusion and ischemia [74] along with induction of growth factors such as vascular endothelial growth factor (VEGF), result in angiogenesis and neovascularization. AGEs can induce crosslinking of collagen which can cause vascular stiffening and entrapment of low-density lipoprotein particles (LDL) in the artery walls. AGEs can also cause glycation of LDL which can promote its oxidation. Oxidized LDL is one of the major factors in the development of atherosclerosis.
- Finally, AGEs can bind to RAGE (receptor for advanced glycation end products) and cause oxidative stress as well as activation of inflammatory pathways in vascular endothelial cells.

AGE and Muller's cells dysfunction :

Winkler et al. have demonstrated that Müller cells exposed to high glucose conditions in vitro produce excess lactate, indicative of increased glycolytic flux, and this effect leads to increased production of MGO.

MC show dysfunction during the hyperglycemia and hypoxia experienced by diabetic retina. This dysfunction is manifested by increased expression of glial fibrillary acidic protein (GFAP), NO production, and concomitant synthesis of glutamate (as a function of disruption of the glutamate transporter which may contribute to excitotoxicity in retinal neurons. 64 Significantly, to greater production of MGO

AGEs and Pericytes dysfunction :

AGEs induce toxic effects on retinal pericytes by causing oxidative stress and subsequent apoptosis. In addition, some studies have indicated that AGEs cause osteoblastic differentiation and calcification in retinal pericytes by the activation of alkaline phosphatases. Pericytes growing on AGE-modified basement membrane show acute attenuation of endothelin-1 (ETA receptor-mediated) contraction, suggesting that AGE cross-linking in a surrounding matrix significantly influences pericyte physiology. Indeed, longer exposure times to these substrate AGEs induce loss of integrin signalling and apoptosis.

The role of AGE triggered inflammation:

Galectin-1 is a hypoxia-induced angiogenic factor associated with proliferative DR. phosphorylation of VEGFR2 and activating its downstream signal transduction in endothelial cells, promotes hypoxia-induced angiogenesis. [76] together with VEGFR1-mediated vascular hyperpermeability [77] Significant up regulation of galectin-1 in eyes of DR patients as disease progressed. AGE stimulation induced the expression of IL-1 β through TLR4 activation and its downstream NF- κ B, ERK1/2 and JNK signal transduction in human monocyte-derived macrophages

Role of RAGEs(AGE receptors)

AGE receptor binding can initiate important signalling pathways involving

- 1) tyrosine phosphorylation of Janus kinase (JAK)/signal transducers and activators of transcription (STAT). [78]
- 2) recruitment of phosphatidylinositol 3' kinase to Ras[37][79]
- 3) activation of protein kinase C.[80]
- 4) and oxidative stress through NF κ B and AP-1 transcription. [81]
- 5) AGEs also inhibit prostacyclin production and stimulate plasminogen activator inhibitor-1 (PAI-1) through an interaction with RAGE) [82]
- 6)AGE-modified serum proteins, such as CML, may interact with vascular endothelium via RAGE, which can activate nuclear factor kappa B (NF- κ B), leading to enhanced expression of adhesion molecules and secretion of cytokines such as tumor necrosis factor alpha (TNF-alpha) and VEGF. Similarly sedentary cells like endothelium encounter AGEs such as pentosidine-derived cross-links within basement membrane proteins where they may disrupt integrin signaling. These findings suggest that AGEs may modify structure and function of target molecules in such a way that they do not respond anymore to biological signals.

Hyperglycemia



AGE



Fig 4.Mechanism of action of AGEs

Source: *Scientific Reports* volume 7, Article number: 16168 (2017)

schema showing the involvement of AGE-triggered inflammatory cues linking to galectin-1 upregulation in the pathogenesis of DR. AGE accumulation due to prolonged hyperglycemia triggers cellular (macrophage) and molecular (IL-1 β) inflammatory cues linking to glial galectin-1 production . Galectin-1

Role of AGE intermediaries in causing DR :

Methyl glyoxal (MG) : glycolysis inevitably produces methylglyoxal (MG) as a by-product. The accumulation of the cell-permeant MG is highly deleterious, since this compound is one of the most potent glycating agents produced in cells.

1) It readily reacts with proteins, lipids and nucleic acids to form

advanced glycation end products (AGEs)

2) Methylglyoxal greatly enhances apoptosis caused by agents that induce oxidative stress and DNA damage.

3) increased modification of vascular basement membrane type IV collagen by methylglyoxal, at hot spot modification sites in the RGD and GFOGER integrin-binding sites of collagen, causes endothelial cell detachment and inhibition of angiogenesis.

4) In addition, matrix glycation impairs agonist-induced Ca²⁺ increases, and this may adversely affect regulatory functions of the endothelium.

5) Aminoacetone also rapidly enolizes and thus readily autoxidizes catalyzed by transition metals to form enolyl radicals, ROS, and iminoacetone. The latter hydrolyzes to form methylglyoxal and NH₄⁺.

6) Aminoacetone also reduces and releases Fe²⁺ from mitochondrial ferritin thereby causing mitochondrial oxidative stress.

7) Mitochondrial succinate-induced respiration was also inhibited and calcium-dependent mitochondrial membrane permeabilization and swelling occurred. DNA oxidation also occurred.

Aminoacetone accumulated in diabetes

3 Deoxyglucose (3 DG)

-Deoxyglucosone (3DG) is a dicarbonyl sugar that is synthesized through the Maillard reaction, [83] and is metabolized to 3-deoxyfructose and 2-keto-3-deoxygluconic acid. 3DG is a precursor for the formation of advanced glycation end-products (AGEs)

Diabetic humans have elevated levels of 3DG and 3-deoxyfructose (3DF) in plasma and urine as compared with non-diabetic individuals. Development of diabetic complications is accelerated in patients with extremely high levels of 3DG in their serum.

3DG plays a central role in the development of diabetic complications via FN3K action.

3DG induces reactive oxygen species (ROS) that contribute to the development of diabetic complications. [84]

3DG inactivates some of the most important enzymes that protect cells from ROS. For example, glutathione peroxidase, a central antioxidant enzyme that uses glutathione to remove ROS, and glutathione reductase, which regenerates glutathione, are both inactivated by 3DG. [20][85] 3DG induces apoptosis in macrophage-derived cell lines. Accumulation of 3 DG leads to neurotoxicity because of ROS generation. (30)[86]

3DG glycosylates and crosslinks proteins leading to a complex mixture of compounds called advanced

glycation end-products (AGEs).[87] AGEs contribute to the diabetic complications including retinopathy . [88]Aminoacetone as a source of ROS, cytotoxicity, and Genotoxicity has been reviewed recently (Bechara et al. 2007).

Biochemical pathways involved in DR:

At least 4 biochemical pathways are involved in DR.

- ❖ The Polyol pathway,
- ❖ The Hexosamine pathway ,
- ❖ The Diacylglycerol -PKC pathway and the
- ❖ The AGE pathway, have already been mentioned above.

Integration of the above pathways into single operating mechanism in DM2 : (Fig)

- **ROS as a single unifying factor behind operation of the pathways causing DR:**
It is well known that ROS is generated in DM2. ROS may activate aldose reductase, induce diacylglycerol, activate PKC, induce advanced glycation end product formation, and activate the pleiotropic transcription factor nuclear factor-kappa B (NF-kappaB). These data demonstrate that a single unifying mechanism of induction, increased production of ROS, serves as a causal link between elevated glucose and each of the three major pathways responsible for diabetic damage. How the 4 pathways cause DR at the nuclear level, these pathways increase histone acetylation through p300 and activate transcription factors upregulating gene expression of vasoactive factors and extracellular matrix (ECM) proteins^{5,6}. These molecules include, endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), fibronectin (FN) and its splice variant extra domain B containing FN (EDB+FN), collagen etc^{5,6}. It is of further interest that there are regulatory relationships among these vasoactive factors and ECM proteins^{7,8}. Among these, ECM protein EDB+FN is absent in normal mature adult tissues, but is expressed in diabetes⁹.
- **Interpretation of mechanism of action of Benfotiamine:**
Benfotiamine is a synthetic lipid soluble analog of thiamine (vitamin B1) which is shown to suppress all these pathways , an unified mechanism was suggested as early as 2003. By inhibition of AR and stimulation of Transketolase enzyme , benfotiamine acted inhibiting the production of sorbitol by polyol pathway and increased the glycolytic flux through transketolase, the key enzyme of HMP shunt and thereby increasing the products- F6P and G3P and consequently increasing the flux through glycolytic pathway .The later happening is doubtful as the very end products of HMP pathway can initiate these pathogenic pathways as shown in fig-.

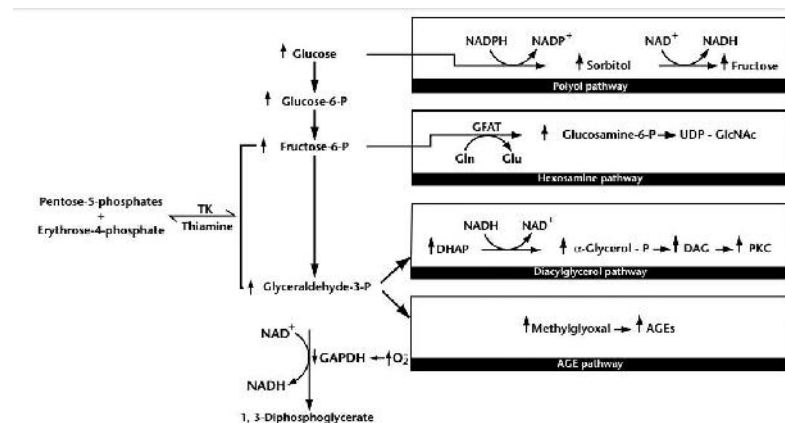


Fig 5. showing the polyol,hexosamine,AGEand PKC pathways.

The Polyol pathway.:

Aldose reductase (AR), the first and rate-limiting enzyme in the polyol pathway reduces glucose to sorbitol using NADPH as a cofactor; sorbitol is then metabolized to fructose by sorbitol dehydrogenase, (SDH) which uses NAD⁺ as a cofactor
In normal physiological conditions, the affinity of hexokinase to glucose is high and hence the amount of sorbitol formed is low. But under conditions of hyperglycaemia, AR is highly active and sorbitol produced is considerable.

increased polyol pathway flux may act through

- decreased (Na⁺&K⁺) ATPase activity,
- sorbitol-induced osmotic stress,
- decrease in cytosolic NADPH,
- and increase in cytosolic NADH/NAD⁺

Role of polyol pathway in DR

- 1 Sorbitol does not easily diffuse through cell membranes; as a result, it accumulates and causes osmotic damage .[89]
- The fructose produced by the polyol pathway can be phosphorylated to fructose-3-phosphate which in turn can be degraded to 3-deoxyglucosone, both of which are strong

Synthesis of UDP-N-acetylglucosamine

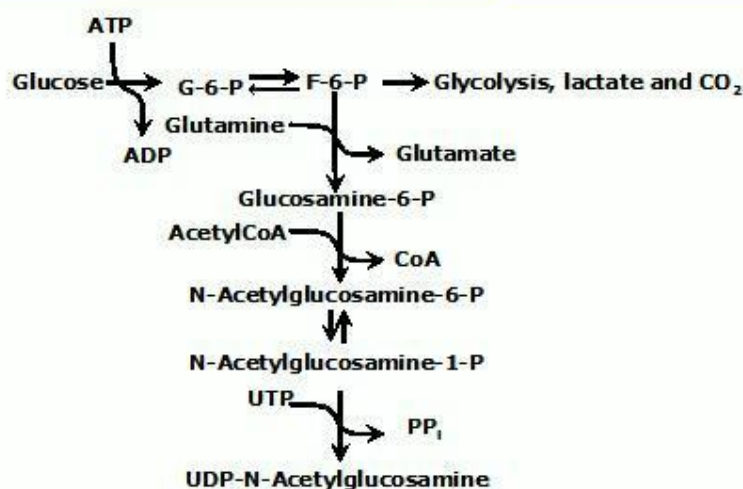


Fig 6 .Showing Hexosamine pathway.

- glycating agents and can result in the production of AGEs [90]
- The use of NADPH as a cofactor in the polyol pathway results in less NADPH availability for use by glutathione reductase, which is crucial for the generation of reduced

glutathione. This decrease in the reduced glutathione available results in a diminished protective response against oxidative stress.[91]

- The aberrant shift of the NADH/NAD⁺ ratio by SDH has been proposed to trigger NADH oxidase which can lead to the increased production of reactive oxygen species (ROS) within the cell .[92]

But therapeutic agents tried against AR yielded mixed results. The long-term sorbinil trial. [93].an SDI, indicated that sorbinil did not prevent the worsening of the disease except for a slower progression rate.

iThe HSP is a relatively minor branch of the glycolytic pathway, encompassing ~ 3% of total glucose utilized .

Increased flux through HBP is required and sufficient for some of the metabolic effects of sustained , increased glucose flux, which promotes the complications of diabetes, *e.g.* diminished expression of sarcoplasmic reticulum Ca²⁺ - ATP-ase in cardiomyocytes and induction of TGF-β and plasminogen activator inhibitor –1 in vascular smooth muscle cells, mesangial cells and aortic endothelial cells. The mechanism was consistent with enhanced O-GlcNAcylation of certain transcription factors. Creased flux of glucose via the Hexosamine pathway has been implicated in insulin resistance, diabetic vascular complications (Figure 2), and stimulation of the synthesis of growth factors increased glucose flux by the Hexosamine pathway may direct retinal neurons to undergo apoptosis in a bimodal fashion, that is, via induction of apoptosis possibly by altered glycosylation of proteins and via perturbation of the neuroprotective effect of insulin mediated by Akt.

The AGE pathway :

Molecular basis of AGE induced DR :

- 1)AGE stimulation induced the expression of IL-1β through TLR4 activation and its downstream NF-κB, ERK1/2 and JNK signal transduction in human monocyte-derived macrophages
- 2) IL-1β acting on retinal Müller glial cells enhanced galectin-1LGALS1 expression via IL1R1 together with intracellular signaling pathways ERK1/2 and PI3K
- 3) galectin-1 interacts with the N-glycans of VEGFR2, to promote hypoxia-induced angiogenesisleading to PDR.
- ,4) Galactun-1, together with VEGFR1- mediates vascular hyperpermeability16. leading to DRM/ NPDR.

The PKC- DAG pathway: (Fig 6)

Synthesis of diacylglycerol begins with glycerol-3-phosphate, which is derived primarily from dihydroxyacetone phosphate, a product of glycolysis (usually in the cytoplasm of liver or adipose tissue cells). Glycerol-3-phosphate is first acylated with acyl-coenzyme A (acyl-CoA) to form lysophosphatidic acid, which is then acylated with another molecule of acyl-CoA to yield phosphatidic acid. Phosphatidic acid is then de-phosphorylated to form diacylglycerol of the microvascular changes in DR are thought to be due to hyperglycemia-induced activation of protein kinase C (PKC)

- 1) The activities of the classical isoforms (PKC-α, -β1/2, and PKC-δ) are greatly enhanced by DAG and have been linked to vascular dysfunctions and pathogenesis of DR([51) [94] the plasma

membrane. [95]

2) PKC activation can lead to mitogen-activated protein kinase (MAPK) activation and phosphorylation of several important transcription factors that increase gene expressions of various stress related genes like c-Jun kinases and heat shock proteins . [96])PKC-b has been shown to have a role in the form of a signalling component for VEGF and a regulator of endothelial cell permeability. [97]

4)PKC activation contributes to the overexpression of plasminogen activator-1 (PAI-1), the activation of NADPH oxidase, and the activation of NFkB in many vascular cells including endothelial cells, smooth muscle cells, pericytes, mesangial cells, and others [65].[98]

PKC Vs AGE;

increased AGEs in retinal vascular endothelial cells contribute to pericyte destruction (Yamagishi et al., 1999), breakdown of the blood-retina barrier and release of PKC (Stitt et al.,1997), providing an important link between the PKC and AGE pathways in the development of retinopathy, and further demonstrating the complexity of these biochemical interactions.

Di acyl glycerol and IR :

Activation of PKC-θ by diacylglycerol may cause insulin resistance in muscle by decreasing IRS1-associated PI3K activity.[8]Similarly, activation of PKCε by diacylglycerol may cause insulin resistance in the liver.

Protein Kinase C Beta in Diabetic Retinopathy

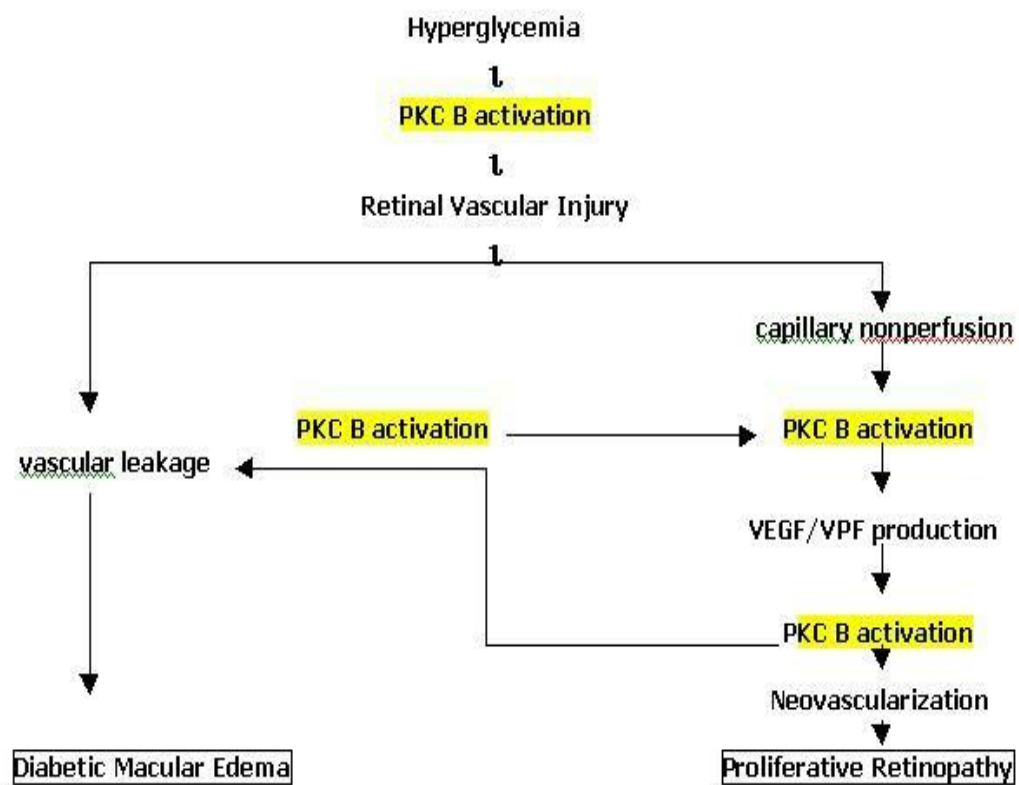


Fig 6. Pkcbeta pathway.

Conclusion:

The complexity of pathogenesis of DR is unveiled. Hyperglycaemia is the ultimate factor inciting each of the factors and pathways involved, individually and collectively. The interventions individually aimed at each of the above mechanisms involved yielded only limited success. A cure for DR is still elusive. The Diabetes Control and Complications Trial (DCCT) and UKPDS were the two landmark clinical trials that showed tight glycaemic control [HbA1c value of 7% or less] could reduce the risk of DR development and progression in T1DM and T2DM patients, respectively.

Randomized controlled trials have shown that early treatment can reduce an individual's risk of severe visual loss by 57%.
intensive glycaemic control) appeared long lasting because of metabolic memory, also known as 'legacy effect'. It is a term used to describe the beneficial effects of immediate intensive treatment of hyperglycaemia with a sustained benefit with respect to the outcomes for many years, regardless of glycaemia in the later course of diabetes.

references final

[1] Klein R, Klein BEK, Moss SE. The Wisconsin epidemiologic study of diabetic retinopathy. IV. Diabetic macular edema. *Ophthalmology*. 1984;91(12):1464–1474. [PubMed]

[2] Yau JW, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. 2012;35(3):556–564. [PMCID: 3312729] [PubMed] 41 [3] Chiang PP

[3] Lamoureux EL, Cheung CY, et al. Racial differences in the prevalence of diabetes but not diabetic retinopathy in a multi-ethnic Asian population. *Investigative ophthalmology & visual science* 2011; 52: 7586–92.

[4] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes care* 2004; 27: 1047–53

[5] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes care* 2004; 27: 1047–53.

[6] Resnikoff S, Pascolini D, Etya'ale D, et al. Global data on visual impairment in the year 2002. *Bulletin of the World Health Organization* 2004; 82: 844–51

[7] Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. 2012;35:556–64. [PMCID: 3312729] [PubMed]

- 895 [8] Bresnick GH: Diabetic retinopathy viewed as a neurosensory disorder. Arch
896 Ophthalmol 104 : 989–990,1986
- 897 [9] Kim JH, Kim JH, Yu YS, Kim DH, Kim KW. Recruitment of pericytes and astrocytes is closely related
898 to the formation of tight junction in developing retinal vessels. J Neurosci Res. 2009;87:653–
899 659. [PubMed]
- 900 [10] Abbott NJ, Rönnebeck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain
901 barrier. Nat Rev Neurosci. 2006;7:41–53. [PubMed]74) . Abbott NJ, Rönnebeck L, Hansson E.
902 Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7:41–
903 53. [PubMed]
- 904 [11] Choi YK, Kim KW. AKAP12 in astrocytes induces barrier functions in human endothelial cells
905 through protein kinase C ζ . FEBS J. 2008;275:2338–2353. [PubMed]
- 906 [12] Cogan D, Toussaint D, Kuwabara T: Retinal vascular pattern. IV. Diabetic
907 retinopathy. Arch Ophthalmol 66:366–378, 1961
- 908 [13] ILP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL
909 3rd, Klein R: Diabetic retinopathy. Diabetes Care 21:143–156, 1998
- 910 [14] Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL
911 3rd, Klein R: Diabetic retinopathy. Diabetes Care 21:143–156, 1998 Astrocyte ref.
- 912 [15] Hohman TC, Nishimura C, Robison WG Jr: Aldose reductase and polyol in
913 cultured pericytes of human retinal capillaries. Exp Eye Res 48:55–60, 1989 100. Nishikiori N, Osanai
914 M, Chiba H, Kojima T, Mitamura Y, Ohguro H, et al. Glial cell-derived cytokines attenuate the
915 breakdown of vascular integrity in diabetic retinopathy. Diabetes. 2007;56:1333–1340. [PubMed]
- 916 [16] Vestweber D. VE-cadherin: the major endothelial adhesion molecule controlling cellular
917 junctions and blood vessel formation. Arterioscler Thromb Vasc Biol. 2008;28:223–232.[PubMed]
- 918 [17] Taddei A, Giampietro C, Conti A, Orsenigo F, Breviario F, Pirazzoli V, et al. Endothelial adherens
919 junctions tight junctions by VE-cadherin-mediated upregulation of claudin-5. Nat Cell
920 Biol. 2008;10:923–934.[PubMed]
- 921
- 922 [18] González-Mariscal L, Betanzos A, Nava P, Jaramillo BE. Tight junction proteins. Prog Biophys Mol
923 Biol. 2003;81:1–44. [PubMed]
- 924
- 925 [19] Wolburg H, Noell S, Mack A, Wolburg-Buchholz K, Fallier-Becker P. Brain endothelial cells and
926 the glio-vascular complex. Cell Tissue Res. 2009;335:75–96. [PubMed]
- 927
- 928 [20] Sawada N, Murata M, Kikuchi K, Osanai M, Tobioka H, Kojima T, et al. Tight junctions and human
929 diseases. Med Electron Microsc. 2003;36:147–156. [PubMed]
- 930
- 931 [21] Dejana E, Giampietro C. Vascular endothelial-cadherin and vascular stability. Curr Opin
932 Hematol. 2012;19:218–223. [PubMed]
- 933 [22] Cui Y, Xu X, Bi H, Zhu Q, Wu J, Xia X, et al. Expression modification of uncoupling proteins and
934 MnSOD in retinal endothelial cells and pericytes induced by high glucose: The role of reactive oxygen
935 species in diabetic retinopathy. Exp Eye Res. 2006;83:807–816. [PubMed]
- 936

- 937 [23] Trudeau K, Molina AJ, Guo W, Roy S. High glucose disrupts mitochondrial morphology in retinal
938 endothelial cells: implications for diabetic retinopathy. *Am J Pathol.* 2010;177:447–455. [PMC free
939 article][PubMed]
940
- 941 [24] Du Y, Kowluru A, Kern TS. PP2A contributes to endothelial death in high glucose: Inhibition by
942 benfotiamine. *Am J Physiol Regul Integr Comp Physiol.* 2010;299:R1610–7. [PMC free
943 article] [PubMed]
944
- 945 [25] Leal EC, Aveleira CA, Castilho AF, Serra AM, Baptista FI, Hosoya K, et al. High glucose and
946 oxidative/nitrosative stress conditions induce apoptosis in retinal endothelial cells by a caspase-
947 independent pathway. *Exp Eye Res.* 2009;88:983–991. [PubMed]
948
- 949 [26] Li AF, Roy S. High glucose-induced downregulation of connexin 43 expression promotes
950 apoptosis in microvascular endothelial cells. *Invest Ophthalmol Vis Sci.* 2009;50:1400–
951 1407.[PubMed]
- 952 [27] Spoerri PE, Afzal A, Li Calzi S, Shaw LC, Cai J, Pan H, et al. Effects of VEGFR-1, VEGFR-2, and IGF-
953 IR hammerhead ribozymes on glucose-mediated tight junction expression in cultured human retinal
954 endothelial cells. *Mol Vis.* 2006;12:32–34. [PubMed]
- 955 [28] Bhattacharjee PS, Huq TS, Potter V, Young A, Davenport IR, Graves R, et al. High-glucose-
956 induced endothelial cell injury is inhibited by a Peptide derived from human apolipoprotein E. *PLoS*
957 *One.* 2012;7:e52152. [PMC free article][PubMed]
958
- 959 [29] Tien T, Barrette KF, Chronopoulos A, Roy S. Effects of high glucose-induced CX43 downregu
960 N, Ashenden M, van Weverwijk A, Lax S, Huso DL, Buckley CD, et al. Pericytes promote selective
961 vessel regression to regulate vascular patterning. *Blood.* 2012;120:1516–1527.[PubMed]
962 Tien T, Barrette KF, Chronopoulos A, Roy S. Effects of high glucose-induced CX43 downregulation on
963 occludin and ZO-1 expression and tight junction barrier function in retinal endothelial cells. *Invest*
964 *Ophthalmol Vis Sci.* 2013;54:6518–6525.[PMC free article] [PubMed]
- 965 [30] Huang Q, Sheibani N. High glucose promotes retinal endothelial cell migration through
966 activation of Src, PI3K/Akt1/eNOS, and ERKs. *Am J Physiol Cell Physiol.* 2008;295:C1647–
967 C1657. [PMC free article] [PubMed]
968
- 969 [31]Yuan L, Hu J, Luo Y, Liu Q, Li T, Parish CR, et al. Upregulation of heparanase in high-glucose-
970 treated endothelial cells promotes endothelial cell migration and proliferation and correlates with
971 Akt and extracellular-signal-regulated kinase phosphorylation. *Mol Vis.* 2012;18:1684–1695. [PMC
972 free article][PubMed]
973
- 974 [32]Su X, Sorenson CM, Sheibani N. Isolation and characterization of murine retinal endothelial
975 cells. *Mol Vis.* 2003;9:171–178.
976 100. Nishikiori N, Osanai M, Chiba H, Kojima T, Mitamura Y, Ohguro H, et al. Glial cell-derived
977 cytokines attenuate the breakdown of vascular integrity in diabetic
978 retinopathy. *Diabetes.* 2007;56:1333–1340. [PubMed]
- 979 [33] Jain S, Suzuki T, Seth A, Samak G, Rao R (2011) Protein kinase C ζ phosphorylates occludin and
980 promotes assembly of epithelial tight junctions. *Biochem J. England.* pp. 289–299.30.Aveleira CA, Lin CM,
981 Abcouwer SF, Ambrosio AF, Antonetti DA (2010) TNF-alpha signals through PKC ζ /NF-kappaB to

- 982 alter the tight junction complex and increase retinal endothelial cell permeability. Diabetes. United
983 States. pp. 2872–2882.
- 984 [34] Aveleira CA, Lin CM, Abcouwer SF, Ambrosio AF, Antonetti DA (2010) TNF-alpha signals through
985 PKCzeta/NF-kappaB to alter the tight junction complex and increase retinal endothelial cell
986 permeability. Diabetes. United States. pp. 2872–2882.
- 987 [35]Titchenell PM, Lin CM, Keil JM, Sundstrom JM, Smith CD, et al.. (2012) Novel atypical PKC
988 inhibitors prevent vascular endothelial growth factor-induced blood-retinal barrier dysfunction.
989 Biochem J. England. pp. 455–467.32.
- 990 [36] Willis CL, Meske DS, Davis TP (2010) Protein kinase C activation modulates reversible increase
991 in cortical blood-brain barrier permeability and tight junction protein expression during hypoxia and
992 posthypoxic reoxygenation. J Cereb Blood Flow Metab. United States. pp. 1847–1859.33.
- 993 2)[37]Barton WA, Tzvetkova D, Nikolov DB (May 2005). "Structure of the angiopoietin-2 receptor
994 binding domain and identification of surfaces involved in Tie2 recognition". *Structure*. **13** (5): 825–
995 32. doi:10.1016/j.str.2005.03.009. PMID 15893672.
- 996 3)[38]Alves BE, Montalvao SA, Aranha FJ, Siegl TF, Souza CA, Lorand-Metze I, et al.
997 (2010). "Imbalances in serum angiopoietin concentrations are early predictors of septic shock
998 development in patients with post chemotherapy febrile neutropenia". *BMC Infect Dis*. **10**:
999 143. doi:10.1186/1471-2334-10-143. PMC 2890004  PMID 20509945.Scott F. Gilbert (10 April
1000 2010). *Developmental Biology (Loose Leaf)*. Sinauer Associates Incorporated. ISBN 978-0-87893-558-
1001 1.
- 1002 [39]Scott F. Gilbert (10 April 2010). *Developmental Biology (Loose Leaf)*. Sinauer Associates
1003 Incorporated. ISBN 978-0-87893-558-1. DM,
- 1004 [40] Griffiths JA, Rojas J, Aldrich TH, Jones PF, Zhou H, McClain J, Copeland NG, Gilbert DJ, Jenkins NA,
1005 Huang T, Papadopoulos N, Maisonpierre PC, Davis S, Yancopoulos GD (Apr 1999). "Angiopoietins 3
1006 and 4: Diverging gene counterparts in mice and humans". *Proc Natl Acad Sci U S A*. **96**(5): 1904–
1007 9. doi:10.1073/pnas.96.5.1904. PMC 26709  PMID 10051567.4)[40]Valenzuela DM, Griffiths JA,
1008 Rojas J, Aldrich TH, Jones PF, Zhou H, McClain J, Copeland NG, Gilbert DJ, Jenkins NA, Huang T,
1009 Papadopoulos N, Maisonpierre PC, Davis S, Yancopoulos GD (Apr 1999). "Angiopoietins 3 and 4:
1010 Diverging gene counterparts in mice and humans". *Proc Natl Acad Sci U S A*. **96**(5)
1011
1012
- 1013 [40]Sarah Y. Yuan; Robert R. Rigor (30 September 2010). *Regulation of Endothelial Barrier*
1014 *Function*. Morgan & Claypool Publishers. ISBN 978-1-61504-120-6.
- 1015
- 1016 [41]Jeansson M, Gawlik A, Anderson G, Li C, Kerjaschki D, Henkelman M, et al. (2011). "Angiopoietin-1
1017 is essential in mouse vasculature during development and in response to injury". *J Clin*
1018 *Invest*. **121** (6): 2278–89. doi:10.1172/JCI46322. PMC 3104773  PMID 21606590
- 1019
- 1020 [42] Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, et al. Molecular definitions of cell
1021 death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell
1022 Death Differ. 2012;19:107–120. [PMC free article][PubMed]
- 1023 [43] Wajant H. The Fas signaling pathway: more than a paradigm. Science. 2002;296:1635–
1024 1636. [PubMed].

- 1025 [44] Boldin MP, Mett IL, Varfolomeev EE, Chumakov I, Shemer-Avni Y, et al. Self-association of the
1026 "death domains" of the p55 tumor necrosis factor (TNF) receptor and Fas/APO1 prompts signaling
1027 for TNF and Fas/APO1 effects. *J Biol Chem.* 1995;270:387–391.[[PubMed](#)]
- 1028 [45] Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Litwack G, Alnemri ES. Molecular ordering of
1029 the Fas-apoptotic pathway: the Fas/APO-1 protease Mch5 is a CrmA-inhibitable protease that
1030 activates multiple Ced-3/ICE-like cysteine proteases. *Proc Natl Acad Sci U S A.* 1996;93:14486–
1031 14491.[[PMC free article](#)] [[PubMed](#)]
- 1032 .[46] Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, et al. Cytochrome c and dATP-
1033 dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease
1034 cascade. *Cell.* 1997;91:479–489. [[PubMed](#)]
- 1035 .[47] Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via
1036 specific labeling of nuclear DNA fragmentation. *J Cell Biol.* 1992;119:493–501. [[PMC free](#)
1037 [article](#)][[PubMed](#)]
- 1038 [48] 36Sun J.K., Lin M.M., Lammer J., Prager S., Sarangi R., Silva P.S., Aiello L.P. Disorganization of
1039 the retinal inner layers as a predictor of visual acuity in eyes with center-involved diabetic macular
1040 edema. *JAMA Ophthalmol.* 2014;132:1309–1316. doi:
1041 10.1001/jamaophthalmol.2014.2350.[[PubMed](#)] [[Cross Ref](#)]
- 1042 [49] Jialal I., Kaur H. The role of toll-like receptors in diabetes-induced inflammation: Implications for
1043 vascular complications. *Curr. Diabetes Rep.* 2012;12:172–179. doi: 10.1007/s11892-012-0258-
1044 7. [[PubMed](#)][[Cross Ref](#)]
- 1045 [50] Serhan C.N., Brain S.D., Buckley C.D., Gilroy D.W., Haslett C., O'Neill L.A., Perretti M., Rossi A.G.,
1046 Wallace J.L. Resolution of inflammation: State of the art, definitions and terms. *FASEB*
1047 *J.* 2007;21:325–332. doi: 10.1096/fj.06-7227rev. [[PMC free article](#)] [[PubMed](#)][[Cross Ref](#)]3DG ref
- 1048
- 1049 .[51] Talahalli R, Zarini S, Tang J, Li G, Murphy R, Kern TS, et al. Leukocytes regulate retinal capillary
1050 degeneration in the diabetic mouse via generation of leukotrienes. *J Leukoc Biol.* 2013;93:135–
1051 143. [[PMC free article](#)][[PubMed](#)]
- 1052 [52] Talahalli R, Zarini S, Tang J, Li G, Murphy R, Kern TS, et al. Leukocytes regulate retinal capillary
1053 degeneration in the diabetic mouse via generation of leukotrienes. *J Leukoc Biol.* 2013;93:135–
1054 143. [[PMC free article](#)][[PubMed](#)]20. Dagher Z, Park YS, Asnaghi V, Hoehn T, Gerhardinger C, Lorenzi
1055 M. Studies of rat and human retinas predict a role for the polyol pathway in human diabetic
1056 retinopathy. *Diabetes.* 2004;53(9):2404–2411. [[PubMed](#)]
- 1057 [53] Zheng L, Du Y, Miller C, Gubitosi-Klug RA, Ball S, Berkowitz BA, et al. Critical role of inducible
1058 nitric oxide synthase in degeneration of retinal capillaries in mice with streptozotocin-induced
1059 diabetes. *Diabetologia.* 2007;50:1987–1996. [[PubMed](#)]21. Gabbay KH. The sorbitol pathway and the
1060 complications of diabetes. *The New England Journal of Medicine.* 1973;288(16):831–836. [[PubMed](#)]
- 1061)54] Krady JK, Basu A, Allen CM, Xu Y, LaNoue KF, Gardner TW, et al. Minocycline reduces
1062 proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent
1063 model of diabetic retinopathy. *Diabetes.* 2005;54:1559–1565. [[PubMed](#)]22. Oates PJ. Polyol pathway
1064 and diabetic peripheral neuropathy. *International Review of Neurobiology.* 2002;50:325–
1065 392. [[PubMed](#)]

- 1066 [55] Yang W, Yu X, Zhang Q, Lu Q, Wang J, Cui W, et al. Attenuation of streptozotocin-induced
1067 diabetic retinopathy with low molecular weight fucoidan via inhibition of vascular endothelial
1068 growth factor. *Exp Eye Res.* 2013;115:96–105. [PubMed]
- 1069 [56] Hu Y, Chen Y, Ding L, He X, Takahashi Y, Gao Y, et al. Pathogenic role of diabetes-induced PPAR-
1070 a down-regulation in microvascular dysfunction. *Proc Natl Acad Sci USA.* 2013;110:15401–
1071 15406.[PMC free article] [PubMed]25) *Diabetes Mellitus: A Fundamental and Clinical*
1072 *Text.* Philadelphia, Pa, USA: Lippincott Williams & Wilkins; 2000.
- 1073 [57] Liu X, Chen HH, Zhang LW. Potential therapeutic effects of pigment epithelium-derived factor
1074 for treatment of diabetic retinopathy. *Int J Ophthalmol.* 2013;6:221–227.[PMC free
1075 article] [PubMed]
- 1076
1077 [58] Yoshida Y, Yamagishi S, Matsui T, Jinnouchi Y, Fukami K, Imaizumi T, et al. Protective role of
1078 pigment epithelium-derived factor (PEDF) in early phase of experimental diabetic
1079 retinopathy. *Diabetes Metab Res Rev.* 2009;25:678–686. [PubMed]Gabbay KH. The sorbitol pathway
1080 and the complications of diabetes. *The New England Journal of Medicine.* 1973;288(16):831–
1081 836. [PubMed]
- 1082 [59] Sorenson CM, Wang S, Gendron R, Paradis H, Sheibani N. Thrombospondin-1 deficiency
1083 exacerbates the pathogenesis of diabetic retinopathy. *J Diabetes Metab.* 2013;(Suppl
- 1084 .[60] Baynes JW. Role of oxidative stress in development of complications in
1085 diabetes. *Diabetes.* 1991;40:405–412.[PubMed]
- 1086 [61] Li J, Wang JJ, Yu Q, Chen K, Mahadev K, Zhang SX. Inhibition of reactive oxygen species by
1087 Lovastatin downregulates vascular endothelial growth factor expression and ameliorates blood-
1088 retinal barrier breakdown in db/db mice: Role of NADPH oxidase 4. *Diabetes.* 2010;59:1528–
1089 1538. [PMC free article][PubMed]
- 1090 [62] Al-Shabrawey M, Rojas M, Sanders T, Behzadian A, El-Remessy A, Bartoli M, et al. Role of
1091 NADPH oxidase in retinal vascular inflammation. *Invest Ophthalmol Vis Sci.* 2008;49:3239–
1092 3244.[PMC free article] [PubMed]
- 1093 [63] Warboys CM, Toh HB, Fraser PA. Role of NADPH oxidase in retinal microvascular permeability
1094 increase by RAGE activation. *Invest Ophthalmol Vis Sci.* 2009;50:1319–1328. [PubMed].
- 1095 [64] Pricci F, Leto G, Amadio L, Iacobini C, Cordone S, Catalano S, et al. Oxidative stress in diabetes-
1096 induced endothelial dysfunction involvement of nitric oxide and protein kinase C. *Free Radic Biol*
1097 *Med.* 2003;35:683–694.[PubMed]
- 1098 [65] Zheng Z, Chen H, Li J, Li T, Zheng B, Zheng Y, et al. Sirtuin 1-mediated cellular metabolic memory
1099 of high glucose via the LKB1/AMPK/ROS pathway and therapeutic effects of
1100 metformin. *Diabetes.* 2012;61:217–228.[PMC free article] [PubMed]
- 1101 [66]Du Y, Veenstra A, Palczewski K, Kern TS. Photoreceptor cells are major contributors to diabetes-
1102 induced oxidative stress and local inflammation in the retina. *Proc Natl Acad Sci*
1103 *USA.* 2013;110:16586–16591.[PMC free article] [PubMed]

- 1104 [67]Gao BB, Clermont A, Rook S, et al. Extracellular carbonic anhydrase mediates hemorrhagic
1105 retinal and cerebral vascular permeability through prekallikrein activation. *Nature*
1106 *Medicine*. 2007;13(2):181–188
- 1107 [68] Weiwei Z, Hu R. Targeting carbonic anhydrase to treat diabetic retinopathy: emerging evidences
1108 and encouraging results. *Biochemical and Biophysical Research Communications*. 2009;390(3):368–
1109 371Wilkinson-Berka JL. Angiotensin and diabetic retinopathy. *International Journal of Biochemistry*
1110 *and Cell Biology*. 2006;38(5-6):752–765.[\[PubMed\]](#)
- 1111 [69]Wilkinson-Berka JL. Angiotensin and diabetic retinopathy. *International Journal of Biochemistry*
1112 *and Cell Biology*. 2006;38(5-6):752–765.
- 1113 [70] Funatsu H, Yamashita H, Nakanishi Y, Hori S. Angiotensin II and vascular endothelial growth
1114 factor in the vitreous fluid of patients with proliferative diabetic retinopathy. *British Journal of*
1115 *Ophthalmology*. 2002;86(3):311–315. [\[PMC free article\]](#)[\[PubMed\]](#)
- 1116 [71] Ebrahimian TG, Tamarat R, Clergue M, Duriez M, Levy BI, Silvestre JS. Dual effect of
1117 angiotensin-converting enzyme inhibition on angiogenesis in type 1 diabetic mice. *Arteriosclerosis,*
1118 *Thrombosis, and Vascular Biology*. 2005;25(1):65–70.
- 1119 [72]Chappey O, Dosquet C, Wautier M-P, et al. Advanced glycation end products, oxidant stress and
1120 vascular lesions. *Eur J Clin Invest*. 1997;27:97–108. doi: 10.1046/j.1365-
1121 2362.1997.710624.x.[\[PubMed\]](#) [\[Cross Ref\]](#)
- 1122 [73]. Vlassara H, Baucala R, Striker L. Pathogenic effects of advanced glycosylation: biochemical,
1123 biological, and chemical implications for diabetes and aging. *Lab Invest*. 1994;70:138–151.[\[PubMed\]](#)
- 1124 .[74]Stitt AW, Li YM, Gardiner TA, et al. Advanced glycated end-products(AGE) co-localise with AGE
1125 receptors in the retinal vasculature of diabetic and AGE infused rats. *Am J Pathol*. 1997;150:523–
1126 539. [\[PMC free article\]](#)[\[PubMed\]](#)
- 1127 [75] Croci, D. O. et al. Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in
1128 anti-VEGF refractory tumors. *Cell* 156, 744–758 (2014).
1129
- 1130 [76]Kanda, A., Noda, K., Saito, W. & Ishida, S. Aflibercept Traps Galectin-1, an Angiogenic Factor
1131 Associated with Diabetic Retinopathy. *Sci Rep* 5, 17946 (2015).
1132
- 1133 [77]Wu, M. H. et al. 9-1 induces vascular permeability through the neuropilin-1/vascular endothelial
1134 growth factor receptor-1 complex. *Angiogenesis*17, 839–849 (2014)
- 1135 [78] Mokini Z, Marcovecchio ML, Chiarelli F. Molecular pathology of oxidative stress in diabetic
1136 angiopathy: role of mitochondrial and cellular pathways. *Diabetes Research and Clinical*
1137 *Practice*. 2010;87(3):313–321.[\[PubMed\]](#)
- 1138 [79] Kowluru RA, Kowluru V, Xiong Y, Ho Y. Overexpression of mitochondrial superoxide dismutase in
1139 mice protects the retina from diabetes-induced oxidative stress. *Free Radical Biology and*
1140 *Medicine*. 2006;41(8):1191–1196.[\[PubMed\]](#)
- 1141 [80]. Kowluru RA, Engerman RL, Case GL, Kern TS. Retinal glutamate in diabetes and effect of
1142 antioxidants. *Neurochemistry International*. 2001;38(5):385–390. [\[PubMed\]](#)

- 1143 .[81] Chan P, Kanwar M, Kowluru RA. Resistance of retinal inflammatory mediators to suppress after
1144 reinstitution of good glycemic control: novel mechanism for metabolic memory. *Journal of Diabetes*
1145 *and its Complications*. 2010;24(1):55–63. [\[PMC free article\]](#) [\[PubMed\]](#)
- 1146 [82]. Menè P, Pascale C, Teti A, Bernardini S, Cinotti GA, Pugliese F. Effects of advanced glycation end
1147 products on cytosolic Ca^{2+} signaling of cultured human mesangial cells. *Journal of the American*
1148 *Society of Nephrology*. 1999;10(7):1478–1486.
- 1149 [83] Maillard (1912). "Action des acides aminés sur les sucres: formation des mélanoidines
1150 par voie méthodique". *Comptes Rendus de l'Académie des Sciences*. **154**: 66–68.
- 1151 [84] Araki A (September 1997). "[Oxidative stress and diabetes mellitus: a possible role of
1152 alpha-dicarbonyl compounds in free radical formation]". *Nihon Ronen Igakkai Zasshi*.
1153 *Japanese Journal of Geriatrics*. (9): 716–20. [PMID 9430981](#).
- 1154 [85]Vander Jagt DL, Hunsaker LA, Vander Jagt TJ, Gomez MS, Gonzales DM, Deck LM, Royer RE (April
1155 1997). "Inactivation of glutathione reductase by 4-hydroxynonenal and other endogenous
1156 aldehydes". *Biochemical Pharmacology*. **53** (8): 1133–40. [doi:10.1016/S0006-2952\(97\)00090-](#)
1157 [7](#). [PMID 9175718](#).Niwa T, Tsukushi S (February 2001). "3-deoxyglucosone and AGEs in uremic
1158 complications: inactivation of glutathione peroxidase by 3-deoxyglucosone". *Kidney International*
1159 *Supplement*. **78**: S37–41. [doi:10.1046/j.1523-1755.2001.59780037.x](#). [PMID 11168980](#).
- 1160
- 1161 [86]Baynes JW, Thorpe SR & Murtiashaw MH (1984). "Nonenzymatic glucosylation of lysine residues
1162 in albumin". *Methods in Enzymology*. *Methods in Enzymology*. **106**: 88–98. [doi:10.1016/0076-](#)
1163 [6879\(84\)06010-9](#). [ISBN 978-0-12-182006-0](#). [PMID 6436646](#).
- 1164 [87]Dyer DG, Blackledge JA, Thorpe SR, Baynes JW (June 1991). "Formation of pentosidine during
1165 nonenzymatic browning of proteins by glucose. Identification of glucose and other carbohydrates as
1166 possible precursors of pentosidine in vivo". *The Journal of Biological Chemistry*. **266** (18): 11654–
1167 60. [PMID 1904867](#).
- 1168 [88]Rahbar S, Kumar Yernini K, Scott S, Gonzales N, Lalezari I (September 1999). "Novel inhibitors
1169 of advanced glycation endproducts". *Biochemical and Biophysical Research*
1170 *Communications*. **262** (3): 651–6. [doi:10.1006/bbrc.1999.1275](#). [PMID 10471380](#).
- 1171
- 1172 [89] Gabbay KH. The sorbitol pathway and the complications of diabetes. *The New England Journal*
1173 *of Medicine*. 1973;288(16):831–836. [\[PubMed\]](#)
- 1174 [90]Szwergold BS, Kappler F, Brown TR. Identification of fructose 3-phosphate in the lens of diabetic
1175 rats. *Science*. 1990;247(4941):451–454. [\[PubMed\]](#)
- 1176 .[91]Barnett PA, Gonzalez RG, Chylack LT, Cheng HM. The effect of oxidation on sorbitol pathway
1177 kinetics. *Diabetes*. 1986;35(4):426–432. [\[PubMed\]](#)
- 1178 192]. Lassègue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and
1179 regulation. *American Journal of Physiology*. 2003;285(2):R277–R297. [\[PubMed\]](#)
- 1180 [93] Glover JP, Jacot JL, Basso MD, Hohman TC, Robison WG. Retinal capillary dilation: early
1181 diabetic-like retinopathy in the galactose-fed rat model. *Journal of Ocular Pharmacology and*
1182 *Therapeutics*. 2000;16(2):167–172-):005. doi:104172/2155-6156.S12-005. [PMC free
1183 article][PubMed]v9. Szwergold BS, Kappler F, Brown TR. Identification of fructose 3-phosphate in the
1184 lens of diabetic rats. *Science*. 1990;247(4941):451–454. [\[PubMed\]](#)

- 1185 [94] Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circulation*
1186 *Research*. 2010;107(9):1058–1070. [\[PMC free article\]](#) [\[PubMed\]](#).
- 1187 [95] Du X, Edelstein D, Rossetti L, et al. Hyperglycemia-induced mitochondrial superoxide
1188 overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1
1189 expression by increasing Sp1 glycosylation. *Proceedings of the National Academy of Sciences of the*
1190 *United States of America*. 2000;97(22):12222–12226. [\[PMC free article\]](#) [\[PubMed\]](#)
- 1191 [96] Chen YQ, Su M, Walia RR, Hao Q, Covington JW, Vaughan DE. Sp1 sites mediate activation of the
1192 plasminogen activator inhibitor-1 promoter by glucose in vascular smooth muscle cells. *Journal of*
1193 *Biological Chemistry*. 1998;273(14):8225–8231. [\[PubMed\]](#)
- 1194 [97]. Gerald P, King GL. Activation of protein kinase C isoforms and its impact on diabetic
1195 complications. *Circulation Research*. 2010;106(8):1319–1331. [\[PMC free article\]](#) [\[PubMed\]](#)
1196 .[98] Koya D, King GL. Protein kinase C activation and the development of diabetic
1197 complications. *Diabetes*. 1998;47(6):859–866. [99] *Erion DM, Shulman GI (2010). "Diacylglycerol-*
1198 *mediated insulin resistance". Nature Medicine. 16 (4): 400–402. doi:10.1038/nm0410-*
1199 *400. PMC 3730126* . PMID 20376053.
12001. [100] Petersen MC, Madsen AK, Gassaway BM, Marcel M, Nasiri AR, Butrico G, Marcucci MJ, Zhang
1201 D, Abulizi A, Zhang XM, Philbrick W, Hubbard SR, Jurczak MJ, Samuel VT, Rinehart J, Shulman GI
1202 (2016). "Insulin receptor Thr1160 phosphorylation mediates lipid-induced hepatic insulin
1203 resistance". *Journal of Clinical Investigation*. **126** (11): 4361–
1204 4371. doi:10.1172/JCI86013. PMC 5096902 . PMID 27760050.