

# Microvascular lesions of diabetic retinopathy: clues towards understanding pathogenesis?

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## Abstract

**Retinopathy is a major complication of diabetes mellitus and this condition remains a leading cause of blindness in the working population of developed countries. As diabetic retinopathy progresses a range of neuroglial and microvascular abnormalities develop although it remains unclear how these pathologies relate to each other and their net contribution to retinal damage. From a haemodynamic perspective, evidence suggests that there is an early reduction in retinal perfusion before the onset of diabetic retinopathy followed by a gradual increase in blood flow as the complication progresses. The functional reduction in retinal blood flow observed during early diabetic retinopathy may be additive or synergistic to pro-inflammatory changes, leucostasis and vaso-occlusion and thus be intimately linked to the progressive ischaemic hypoxia and increased blood flow associated with later stages of the disease. In the current review a unifying framework is presented that explains how arteriolar dysfunction and haemodynamic changes may contribute to late stage microvascular pathology and vision loss in human diabetic retinopathy.**

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**Keywords:** diabetes; diabetic retinopathy; blood flow; haemodynamics; hypoxia; ischaemia

## Background

The worldwide incidence of diabetes is set to rise dramatically from the present incidence of 150 million to an estimated 300 million in 2025.<sup>1</sup> Most cases will be of type II diabetes, which is

closely linked to the upsurge in obesity. The complications arising from diabetes impose an ever-increasing burden on health-care authorities in both developed and developing countries. As one of the most common microvascular complications of this disease, retinopathy is set to remain a major clinical issue for the ophthalmologist. Indeed, epidemiological studies indicate that following 20 years of diabetes, nearly all patients with type I diabetes will have at least some retinopathy. Moreover, ~80% of insulin-dependent type II diabetic patients and 50% of type II diabetic patients not requiring exogenous insulin will have retinopathy after 20 year disease duration.<sup>2</sup> The seminal DCCT and UKPDS clinical studies in type I and type II diabetes patients, respectively, have established the relationship between time-averaged levels of glycaemia and progression of retinopathy<sup>3,4</sup> and these have formed a strong foundation for further research seeking to identify the cellular and molecular mechanisms underpinning retinal cell damage in diabetes.

Retinal microvascular pathology is central to understanding the nature of retinal defects during diabetes and is the focus of this review. However, our overview would be incomplete without highlighting that the purpose of these vessels is to support the metabolic demands of the inner retinal neurons and glia. Nowhere is this more apparent than in the macula, where metabolic demand is highest and the microvascular tree is at its most dense (Figure 1). Indeed, growing evidence indicates that a significant neuroglial dysfunction also occurs during diabetic retinopathy in unison with blood flow abnormalities, and before the appearance of microvascular lesions (reviewed by Antonetti *et al*<sup>5</sup>). In diabetic patients and

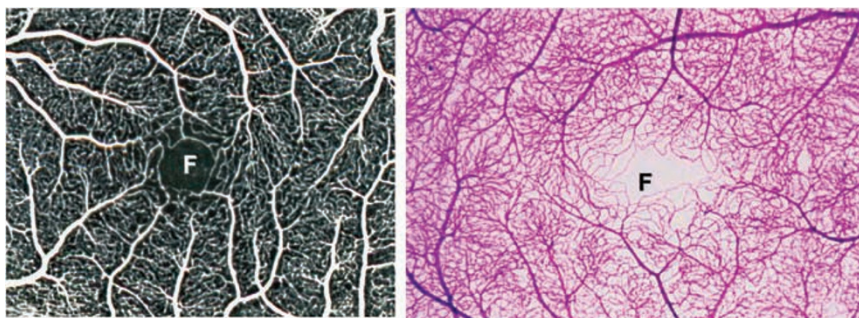
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**Figure 1** A fluorescein angiogram of the left macula demonstrating the perfusion density adjacent to the avascular foveola (F). A trypsin digest preparation from a corresponding region shows the capillary density in even more detail and also highlights the clear benefit of this histological preparation for evaluating the retinal microvasculature.

short-term animal models of diabetes reversible alterations are evident in the electroretinogram (such as loss of oscillatory potentials<sup>6</sup>) together with defects in colour perception<sup>7</sup> and impaired contrast sensitivity.<sup>8</sup> These well-characterized electrophysiological deficits may represent a progressive 'neuropathic' aetiology to diabetic retinopathy.<sup>5</sup> This is supported by detailed post-mortem studies and investigation of animal models. For example, in diabetic rodents there is a perturbation of the retinal histaminergic neurons leading to swelling of centrifugal axons<sup>9</sup> whereas ganglion cells appear to suffer apoptotic death, even after short-term (<4 months) diabetes.<sup>10,11</sup> There is a thinning of the outer nuclear layer as diabetes progresses,<sup>12</sup> loss of dopaminergic neurons,<sup>13</sup> and ~50% depletion of cells in the inner nuclear layer after >4 months experimental diabetes.<sup>14</sup> Glial cells also suffer during hyperglycaemia,<sup>15</sup> in particular the Müller macroglia which demonstrate increased expression of glial fibrillary acidic protein,<sup>16</sup> loss of osmotic balance associated with a decrease in K<sup>+</sup> currents,<sup>17</sup> and concomitant synthesis/release of glutamate (as a function of disruption of the glutamate transporter<sup>18</sup>) which contributes to excitotoxicity in the retinal neuropile.<sup>19</sup>

Although the retina suffers a neuroglial pathology during diabetes, the clinically observed intraretinal microvascular changes serve as the basis for classification into non-proliferative diabetic retinopathy and proliferative diabetic retinopathy. Mild non-proliferative diabetic retinopathy (often referred to as background retinopathy) and severe proliferative retinopathy actually represent the full spectrum of the same disease process. Non-proliferative diabetic retinopathy is characterized by a complex array of vasodegenerative lesions within the retinal microvascular bed, including thickening of capillary basement membranes (BMs), pericyte and vascular smooth muscle cell (VSMC) dropout, microaneurysms, and capillary occlusion and acellularity (for a review see Gardiner *et al*<sup>20</sup>).

Visual impairment normally occurs in the later stages of diabetic retinopathy with the development of macula oedema as a direct consequence of inner blood-retinal barrier (iBRB) breakdown. In the proliferative phase of the disease there is an abnormal growth of new blood vessels (retinal neovascularization) that give rise to sight-threatening vitreous haemorrhage and tractional retinal detachment. Macular oedema and retinal neovascularization occur as a result of increasing inner retinal ischaemia- and hypoxia-driven secretion of cytokines and growth factors, the best known being vascular endothelial growth factor (VEGF). Although sight-threatening diabetic retinopathy can be treated or contained to some extent by pan-retinal laser photocoagulation or vitreoretinal surgery this is often at the expense of functional retina and visual performance. Therefore, there is a genuine and urgent need for effective treatments for all stages of diabetic retinopathy.

#### Abnormal haemodynamics in diabetic retinopathy

The retinal vasculature lacks autonomic innervation and modulation of blood flow through the neuropile is dependant on local signalling mechanisms.<sup>21</sup> As early as the 1930s it was suggested that retinal blood flow was markedly altered in diabetic patients.<sup>22</sup> As studies progressed and technology became ever more sophisticated there were strong indications that retinal vessel calibre consistently increased during diabetes.<sup>23,24</sup> Direct quantification of mean circulation time during fluorescein angiography by Kohner *et al*<sup>25</sup> demonstrated that that retinal blood flow was enhanced in diabetic subjects with absent or mild retinopathy, but not in those with moderate or severe diabetic retinopathy. Since these seminal studies, haemodynamic change in the diabetic retina has been the focus of considerable research although significant discrepancies exist between reported findings. This may be attributable to the variety of techniques used to measure retinal blood flow and also disparities between demographic and metabolic

parameters (blood glucose, lipids, insulin, blood pressure, diabetes duration etc) of the study cohorts. As a synthesis, the majority of studies suggest that patients with short duration diabetes (<5 years) show a constriction of the major arteries and arterioles<sup>26</sup> and retinal blood flow is decreased.<sup>27,28</sup> With more prolonged diabetes duration and concomitant presence of clinical retinopathy, arterial vessels begin to dilate and bulk retinal blood flow increases in proportion with the severity of retinopathy.<sup>29–31</sup> Blood flow changes in diabetes are widely recognized but there is little direct evidence in the literature that such alterations contribute to pathology.

### Retinal hypoperfusion in diabetes: links to early pathology

As outlined above, a decrease in retinal blood flow represents one of the earliest abnormalities observed in the diabetic retina. This has been confirmed in patients with type I diabetes without retinopathy using methods based on video fluorescein angiography.<sup>32,33</sup> Laser Doppler techniques have also revealed reduced blood flow in the large retinal vessels of diabetic patients with no retinopathy.<sup>28</sup> In many cases, this phenomenon is linked to hyperglycaemia and haemoglobin A1c (HbA<sub>1c</sub>) with normal retinal haemodynamics occurring in well-controlled diabetic patients (HbA<sub>1c</sub> ≤7.5%) with no or minimal retinopathy.<sup>34</sup> It is worth noting that comparable dysregulation of retinal blood flow has also been reported in rodents up to 12 weeks after diabetes induction.<sup>35</sup> Such animal models have formed an important basis for understanding the mechanism(s) of diabetes-mediated hypoperfusion in the retina.

Acute or chronic exposure to the diabetic milieu results in a range of biochemical and metabolic abnormalities. As a result of considerable research effort in the past 20 years, many related pathogenic mechanisms have been implicated in the progression of diabetic retinopathy. Many of these pathways are interrelated<sup>36</sup> and it should be appreciated that hyperglycaemia can simultaneously provoke several other pathogenic pathways in retinal cells. One such mechanism is linked to increased flux through the polyol or hexosamine pathways which is associated with subsequent alterations in the redox state of pyridine nucleotides.<sup>37</sup> Accumulation of sorbitol in retinal cells is dependant on the activity of aldose reductase and this may impinge on a range of pathways and contribute to diabetic retinopathy.<sup>38</sup> Also *de novo* synthesis of diacylglycerol (DAG) leading to the over-activation of several isoforms of protein kinase C (PKC),<sup>39</sup> excessive production of free radicals leading to oxidative stress,<sup>40,41</sup> changes in blood rheology and haemodynamics,<sup>42,43</sup> and over-activation of the renin-angiotensin system<sup>44</sup> contribute significantly to

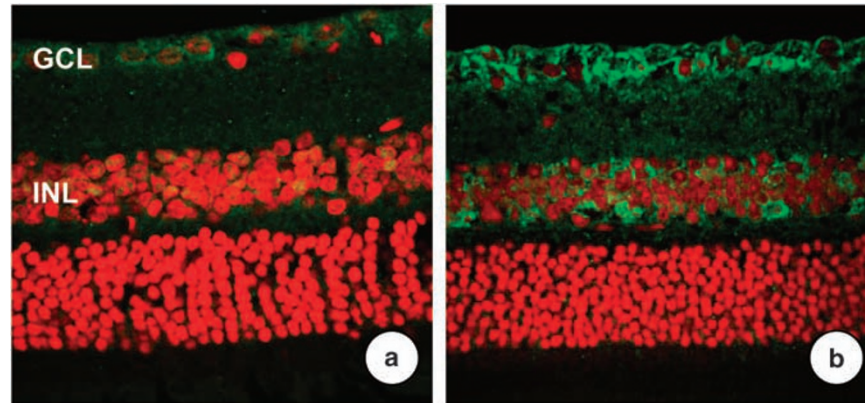
retinopathy as diabetes progresses. Accumulation of advanced glycation end products (AGEs) and activation of receptors for AGEs are also important pathogenic mechanisms with clear links to diabetic retinopathy.<sup>36,45</sup>

With particular links to early changes in retinal blood flow, there has been considerable emphasis placed on the activation of PKC, including several conventional and novel isoforms such as PKC $\alpha$ ,  $\beta$ ,  $\delta$ , and  $\epsilon$ .<sup>46</sup> PKC activation in diabetes may also arise through oxidative stress or increased concentrations of free fatty acids.<sup>47</sup> The PKC $\beta$ II isoform is preferentially activated in diabetic retinopathy and this can be linked to impaired retinal blood flow.<sup>46</sup> Intravitreal injection of a DAG kinase inhibitor (that elevates total retinal DAG levels) or a PKC-activating phorbol ester serves to decrease retinal blood flow in non-diabetic rats.<sup>48</sup> Furthermore, PKC $\beta$  knockout mice rendered diabetic exhibit no abnormalities in retinal blood flow<sup>35</sup> whereas the specific PKC $\beta$  inhibitor ruboxistaurin (previously known as LY333531) improves retinal blood flow in diabetic animals.<sup>49</sup> These and other related findings formed a basis for therapeutic targeting of PKC $\beta$ II using the inhibitor ruboxistaurin in the hope of preventing the progression of diabetic retinopathy. This agent protected against retinal haemodynamic dysfunction in diabetic patients<sup>50</sup> although protection was not observed for some aspects of pathology in a recent clinical trial. Ruboxistaurin did however achieve significant reduction in diabetic macular oedema.<sup>51</sup>

Another major pathway that modulates retinal vasoconstriction and decreased retinal blood flow during early diabetes is disruption of ion channel function. This dysfunction is largely concentrated at the level of the retinal arterioles as the main site for local blood flow regulation in the retina. Retinal arteriolar VSMCs express several classes of plasma membrane ion channels, including voltage-gated K<sup>+</sup> channels, large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels (BK channels), Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels, and L-type Ca<sup>2+</sup> channels which are important in controlling retinal vascular tone and blood flow.<sup>52</sup> Within the context of diabetic retinopathy, the BK channels on retinal arteriolar VSMCs are particularly important because they show reduced Ca<sup>2+</sup> sensitivity during early diabetes and render a sustained vasoconstrictive response when compared to non-diabetic controls.<sup>53</sup> BK channel dysfunction during early diabetic retinopathy may represent a central mechanism underlying the hypoperfusion observed in patients and animal models.

### Hypoperfusion and retinal hypoxia

From an experimental perspective, it can be surmised that deficits in retinal perfusion could impact



**Figure 2** Retinal hypoxia in short-term diabetes. The bio-reductive drug pimonidazole can be introduced to animals after which it forms irreversible, insoluble adducts with thiol groups on tissue proteins when  $PO_2$  is below 10 mm Hg. These adducts can then be immunolocalized using a pimonidazole-adduct antibody. In comparison to non-diabetic control retina (a), diabetic mouse retina (b) (5 months diabetes duration) hypoxyprobe immunoreactivity (green fluorescence) is intense at the level of the ganglion cell layer (GCL) and in cells within the inner nuclear layer (INL).

significantly on the oxygenation of the metabolically demanding neural retina. If this association could be solidified, it would have important implications for our understanding of microvascular and neuroglial pathology during diabetic retinopathy. Deficits in oxygen delivery to the retina are observed in diabetes.<sup>54</sup> Such hypoxia could be linked, at least in part, to blood flow abnormalities as evidenced by the finding that  $PO_2$  in diabetic cats is significantly lower than non-diabetic counterparts, even before capillary dropout is clinically observable.<sup>55</sup> Our studies using the drug pimonidazole (which deposits as immunoreactive insoluble adducts at  $PO_2 < 10$  mm Hg) has also demonstrated that hypoxia occurs in relatively short-term diabetes in mice (Figure 2). Furthermore, diabetic patients with no retinopathy or minimal lesions breathing pure oxygen show improved contrast sensitivity and colour vision suggesting that tissue hypoxia also occurs early in the course of the human disease.<sup>56,57</sup>

#### *Hypoperfusion and retinal capillary leucostasis*

A well-described phenomenon occurring within weeks of diabetes onset is retinal capillary leucostasis, whereby blood-borne leucocytes adhere strongly to the endothelial plasma membrane and become entrapped, leading to capillary occlusion.<sup>58</sup> Although it is difficult to determine the net contribution of leucostasis to long-term pathology, the early decrease in retinal blood flow during diabetes is likely to promote endothelial–leucocyte interactions and contribute to increased leucostasis. Under such circumstances leucocytes cannot pass unobstructed through capillary beds and they can become lodged in the narrow lumen of the capillary channels. This can result in the blockage of the affected

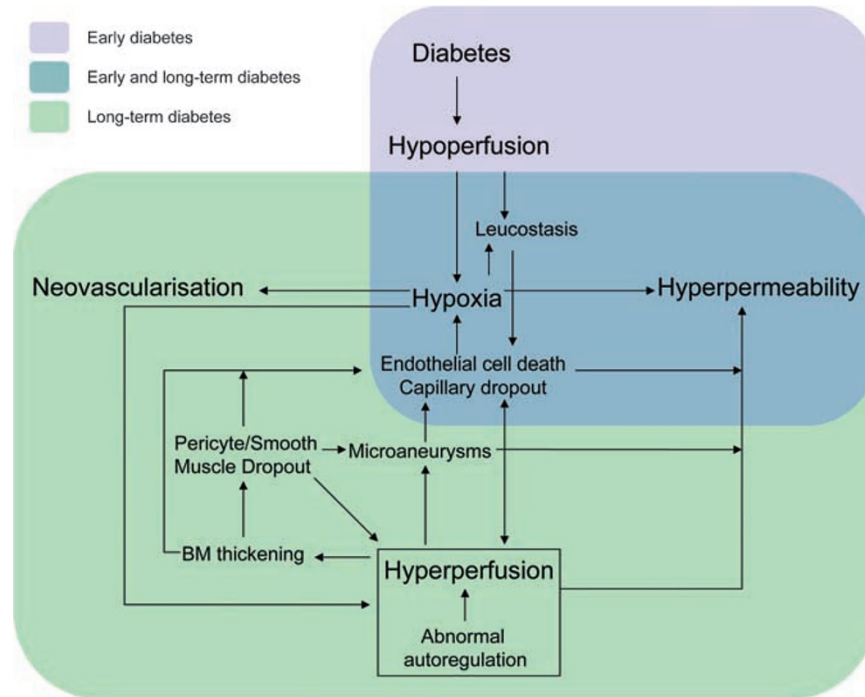
capillary, reducing the blood flow in that area of retina and causing local tissue ischaemia.<sup>58</sup> Leucocytes are less deformable in diabetics and this, in combination with their inappropriate adherence and inherent capacity to generate toxic superoxide radicals,<sup>59,60</sup> has important implications for capillary non-perfusion, endothelial cell damage, and vascular leakage in the retinal microcirculation.<sup>61</sup>

#### *Hypoperfusion and hyperperfusion*

As presented, in the early stages hypoperfusion may contribute to a low-grade, chronic inflammation of the retinal vasculature resulting in capillary dropout and the development of a progressive, irreversible ischaemic hypoxia. Tissue hypoxia causes retinal vasodilatation and enhanced retinal blood flow<sup>52</sup> indicating that the aforementioned switch from hypoperfusion to hyperperfusion during diabetic retinopathy is probably linked to hypoxia. Indeed, it could be speculated that when retinal hypoxia reaches a certain threshold it may override the direct vasoconstrictive effects of diabetes, thereby instigating the shift to retinal hyperperfusion (Figure 3). Among the proposed mechanisms to explain retinal hypoxic vasodilatation are the release of metabolic factors from the surrounding neural tissues and the production of vasoactive agents from the endothelium.<sup>62</sup>

#### **Can retinal hyperperfusion in diabetic retinopathy be linked to microvascular histopathology?**

As diabetic retinopathy progresses there is a shift from hypoperfusion to hyperperfusion and this is associated with the transition to background and pre-proliferative



**Figure 3** A unifying haemodynamic model for the pathogenesis of diabetic retinopathy. Without demonstrating the complexities of metabolic and biochemical pathogenic pathways that superimpose on this model, the purple region shows how early stage hypoperfusion could lead to progressive hypoxia and increased leucocytic adherence to the retinal capillaries. As diabetes develops, the retinal microvasculature shows hyperperfusion leading to BM thickening, loss of arteriolar tone, microaneurysms, and capillary dropout that, in-turn, accelerate the hypoxic insult on the retina.

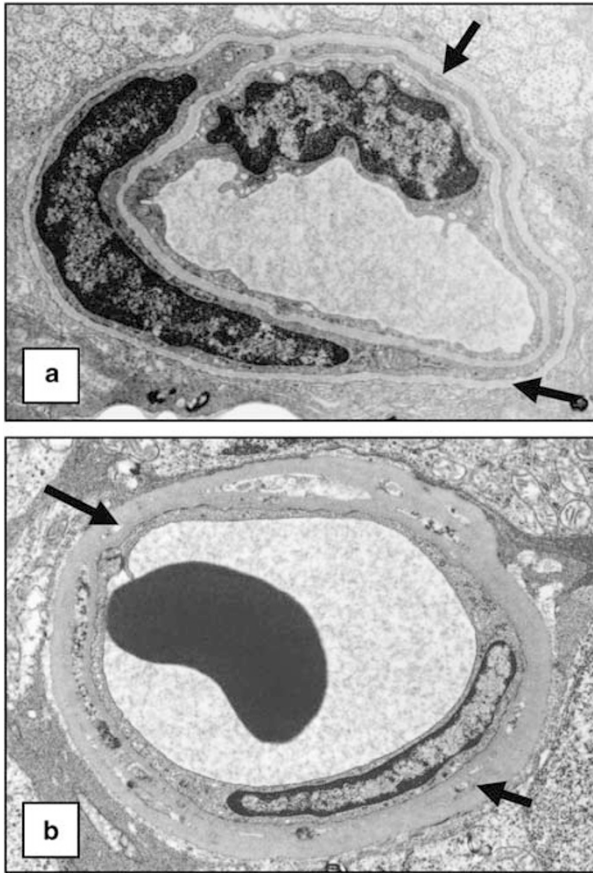
diabetic retinopathy.<sup>30,63</sup> The particular nature of perfusion changes in patients with proliferative retinopathy tend to be associated with the microvascular pathology present in the fundus image. This is evidenced by patients with extensive retinal ischaemia which have lower volumetric flow rates than those patients that retain reasonable capillary viability.<sup>64</sup> These findings may be reconciled on the basis that capillary density is known to be inversely correlated with vascular resistance. Some of the defined microvascular lesions of diabetic retinopathy could be linked to this shift from hypoperfusion to hyperperfusion. Indeed, the Australian Diabetes, Obesity and Lifestyle study actually indicated retinal arteriolar dilatation as a specific and effective indicator of diabetic microvascular dysfunction that could be used as a ‘pre-pathology’ marker for initiation and progression in diabetic retinopathy.<sup>65</sup> This indicates that better-quality imaging, imaging processing, and newer techniques such as hyperspectral retinal imaging may provide tangible ways of predicting risk or monitoring progression of diabetic retinopathy in patients. We have proposed a model to explain how such alterations in retinal blood flow may lead to the initiation and progression of diabetic retinopathy (Figure 3). In particular, an emphasis has been placed on interrelationships between retinal blood flow and the

evolution of the microvascular lesions that are characteristic of this complication.

#### *Retinal capillary basement membrane thickening*

Thickening of the retinal capillary BM represents an important histopathological hallmark of diabetic retinopathy (Figure 4). The expansion and change in protein composition of this specialized extracellular matrix is a reflection of the increased expression of vascular BM component proteins and a net reduction in proteolytic digestion.<sup>20</sup> There is evidence that hyperperfusion could contribute to this lesion because increased flow, in combination with raised blood viscosity, in diabetes causes endothelial responses that increase BM thickness. Shear stress is known to regulate endothelial cell gene expression through activation of multiple intracellular signalling cascades leading to expression of BM component proteins such as collagen IV, laminin, and fibronectin.<sup>66</sup> In fact this represents a physiological response to maintain vascular integrity by bolstering the matrix of the vessel wall. Shear stress levels are greatest in retinal arteries and arterioles<sup>67</sup> and during diabetes BM thickening is significantly increased in capillaries that are located proximal to the arterial side of the retinal circulation.<sup>68</sup>

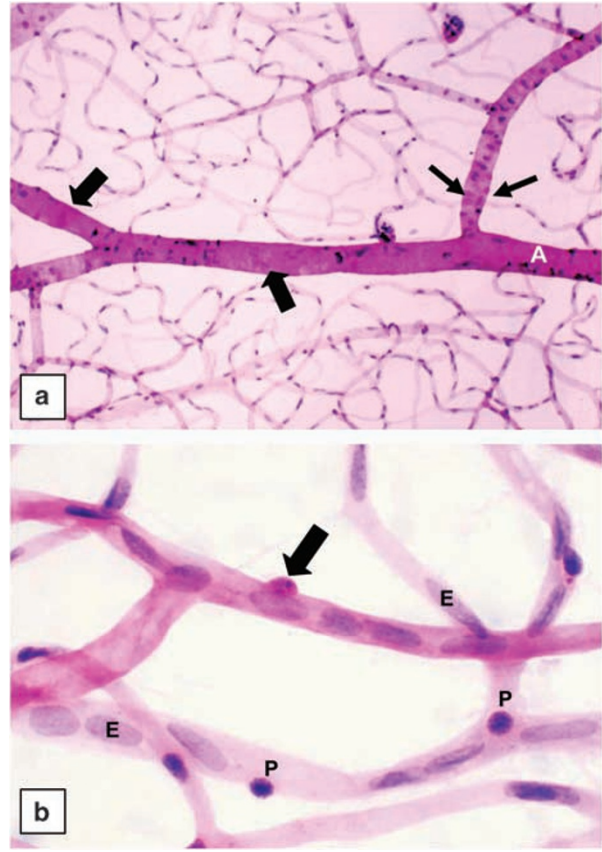




**Figure 4** Vascular basement membrane (BM) thickening in diabetic retinal capillaries. Transmission electron microscopy (TEM) of rat retina shows that retinal capillary BMs (arrows) are susceptible to thickening during 12 months diabetes (compare non-diabetic, a, with diabetic, b).

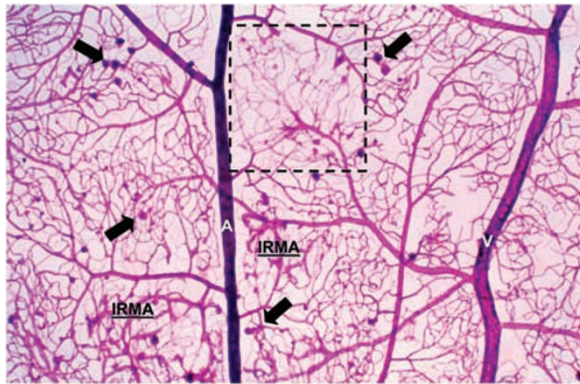
#### Loss of retinal pericytes

In the human retina, pericytes occur in a unique 1:1 ratio with capillary endothelium and loss of these cells during diabetic retinopathy is a characteristic pathology<sup>69</sup> (Figure 5). Progressive pericyte demise in the retina is readily visualized by the trypsin digest technique and so-called pericyte ghosts are actually pockets of cell debris sequestered within the capillary BM. Less well recognized but equally important is the parallel demise of arterial/arteriolar VSMCs (Figure 5) in both animal models and patients.<sup>20</sup> Focal and/or extensive loss of VSMCs has serious implications for arteriolar integrity and autoregulation of blood flow and may severely aggravate the retinal haemodynamic abnormalities observed in long-term diabetes. The mechanism underlying premature death of pericytes and VSMCs during diabetic retinopathy is the subject of significant research effort and a range of pathways have been



**Figure 5** Trypsin digest of retinal vasculature from 5-year diabetic dog shows widespread loss of arteriolar smooth muscle (SM) cells (thick arrows) (a). SM cell 'ghosts' stain red with the periodic acid-Schiff technique (thin arrows). (b) Trypsin digest of retinal vasculature of 5-year diabetic dog. A pericyte 'ghost' (arrow) in the wall of a small venule stains red with the periodic acid-Schiff technique. Normal pericyte (P) and endothelial cell nuclei (E) stain blue with haematoxylin.

identified such as oxidative stress, polyol pathway flux, activation of PKC, and accumulation of advanced AGEs.<sup>70</sup> It is unlikely that pericytes/VSMC loss is directly linked to hyperperfusion; however it could be speculated that vascular BM thickening and alteration in the protein composition and charge selectivity could impact on the survival of these cells within the diabetic milieu. A two-way communication between endothelial cells and pericytes/VSMCs is vital for the maintenance of vessel integrity and function.<sup>71</sup> Endothelial cells release vasoactive agents such as platelet-derived growth factor B for pericyte/VSMC survival,<sup>72</sup> whereas these cells, in turn, express VEGF and angiopoietin-1 that enhance the survival and integrity of the endothelium.<sup>71</sup> It is likely that BM thickening serves to limit communication between endothelial cells and pericytes/VSMCs and thus contributes to accelerated vascular cell death and vessel



**Figure 6** Low-magnification image of a trypsin digest from a type II diabetic patient with long-standing (>15 years) non-proliferative diabetic retinopathy. Note that microaneurysms are predominantly located on capillaries adjacent to precapillary arterioles (arrows). Faintly stained acellular capillaries underlie many regions of the capillary bed, although confluent regions tend to have a peri-arteriolar location and may be bridged by intraretinal microvascular abnormalities (IRMA), as in the lower part of the image or not, as in the boxed region. Artery, A; vein, V.

instability in the diabetic retina. In any case, loss of pericytes and smooth muscle forms a reasonable explanation for why retinal blood flow abnormalities are irreversible in some diabetic patients with established retinopathy.<sup>73</sup>

#### **Endothelial cell death and acellular capillary formation**

Loss of capillaries is a central tenet of progressive ischaemia during diabetic retinopathy and is a universal finding in retina from long-term diabetic animal models and post-mortem specimens.<sup>20</sup> On the trypsin digest, these acellular capillaries appear as non-perfused naked BM tubes where the endothelial cells have disappeared.<sup>20</sup> Diabetes appears to greatly accelerate the turnover and renewal of retinal microvascular endothelial cells<sup>74</sup> and with prolonged diabetes these cells may exhaust their replicative lifespan as they exceed the so-called Hayflick limit.<sup>75</sup> There is evidence that bone-marrow-derived endothelial progenitor cells could contribute to a compromised endothelial monolayer because these cells are also dysfunctional in diabetes,<sup>76</sup> as is the vessel wall they differentiate onto.<sup>77</sup> In combination, these changes eventually lead to the formation of acellular capillaries (Figure 6).

The underlying endothelial cell insult is undoubtedly linked to previously mentioned biochemical dysfunction and evoked pathogenic pathways. However, in addition to these, hyperperfusion and resultant shear stress in the later stages of diabetic retinopathy may directly provoke

retinal endothelial cell death and capillary occlusion.<sup>35</sup> Physiological levels of shear stress are known to suppress endothelial cell apoptosis through activation of pro-survival signalling pathways such as the PI3/Akt pathway<sup>78</sup> although pathologically enhanced shear stress can induce endothelial cell death.<sup>79</sup>

#### **Retinal microaneurysm formation**

Retinal microaneurysms have also been described in non-diabetic conditions (eg hypertension, retinal branch vein occlusions, and leukaemia) but these lesions appear with the greatest frequency in diabetic retinopathy. In fact, from the fundus image perspective, microaneurysms are the earliest clinically recognizable feature of diabetic retinopathy and they have been used as predictive markers for disease progression. Ophthalmoscopically, microaneurysms appear as small, dark red spots, approximately 10–100 μm in diameter whereas on fluorescein angiography they manifest as hyperfluorescent spots that may fade in the later phases of the angiogram. The precise nature of microaneurysms is best appreciated by trypsin digest preparation of post-mortem eyes from diabetic donors and depicts morphological forms ranging from thin walled cellular types to dense, acellular, hyalinized forms<sup>80</sup> (Figure 6). The cellular forms can often include accumulations of monocytes and polymorphonuclear cells.<sup>80</sup>

Loss of pericytes alone cannot account for microaneurysm formation but this feature appears to be critical<sup>80</sup> especially because these structures are often downstream from arteriolar VSMC loss and vasodilatation (Figures 5 and 6). Indeed, the role of intraluminal pressure in the pathogenesis of microaneurysms is supported by the observation that these lesions tend to occur largely on the arterial side of the capillary bed in both diabetic patients and long-term animal models such as diabetic dogs (Figure 6).<sup>20,80</sup> In diabetic patients, microaneurysms tend to cluster upstream of large areas of capillary non-perfusion, indicating a common link to occlusion.<sup>20</sup> Interestingly microaneurysms fail to develop in diabetic rodents. This may be because the rodent retinal vasculature, unlike that in humans and dogs, contains precapillary sphincters<sup>81</sup> which probably protect capillary beds from hypertensive injury.

Geographic variability exists in the distribution of acellular capillaries and microaneurysms within the diabetic retina. In both dogs and humans, capillary acellularity is significantly more prevalent within the temporal retina than in the nasal retina<sup>82</sup> and progresses from the arterial to venous side of the retinal circulation as the disease progresses.<sup>83</sup> Because flow rate in temporal retinal vessels is greater than that in nasal vessels<sup>84</sup> and

flow-related stress is probably enhanced in the juxta-arteriolar capillary beds, this also provides support for the key involvement of haemodynamic dysfunction in diabetic retinopathy.

## Retinal blood flow and vision loss in diabetic retinopathy

### Macular oedema

Fluid balance across the retinal capillary beds depends upon the hydrostatic and oncotic pressure gradients. The hydrostatic pressure gradient within capillaries serves to push fluid out of the vessel into the interstitium. As a counterbalance, the oncotic pressure gradient is formed by protein concentration in the blood column that draws fluid into the capillaries. When the hydrostatic pressure gradient and oncotic pressure gradient are in balance there is no imbalance of fluid movement between the capillaries and the tissue compartment. For the retina, this equilibrium is particularly important because there are no lymphatics present to drain excess fluid from the interstitium and it is critical to maintain fluid balance for neural health and function.

When excessive vasopermeability occurs at the macular, so-called diabetic macular oedema can precipitate serious loss of visual acuity within a short time frame. This pathology can occur quite early, but is most prevalent in the late stages of diabetic retinopathy. The Wisconsin epidemiological study demonstrated that diabetic macular oedema occurred in up to 6% of patients with mild non-proliferative retinopathy but this figure rose dramatically to 20–63% of patients with moderate to severe non-proliferative retinopathy and 70–74% of patients with proliferative retinopathy.<sup>85</sup> In fluorescein angiograms, diabetic macular oedema can appear as focal or diffuse, with the former associated with leaking microaneurysms and the later being a consequence of wholesale breakdown of the iBRB adjacent to the macula.<sup>86</sup> The Early Treatment Diabetic Retinopathy Study demonstrated that focal laser photocoagulation in the macula provides benefit to patients with diabetic macular oedema<sup>87</sup> although this prevented further vision loss rather than to improve vision.

Breakdown of the iBRB can occur early after establishment of diabetes and most research focus has been placed on short-term retinopathy models in rodents where acute-phase vasopermeability is evident after only 2–3 weeks diabetes.<sup>88,89</sup> Various studies have shown that manipulation of adhesion molecules, pro-inflammatory cytokines, and nitric oxide can prevent this lesion within an acute time frame.<sup>88,89</sup> It remains uncertain how iBRB compromise early in diabetes relates to more long-term, sight-threatening macular oedema.

The precise mechanism of iBRB compromise during diabetic retinopathy remains incompletely elucidated but there are firm links with diabetes-mediated upregulation of the potent vasopermeability factor VEGF from the neural retina<sup>90</sup> (Figure 7). VEGF modulates loss of tight junction integrity or enhanced transport mechanisms in endothelial cells in the early stages of diabetic retinopathy.<sup>91,92</sup> Upregulation of this growth factor occurs early in diabetes, which suggests that expression may be linked to acute hyperglycaemia, alterations in retinal blood flow, and/or enhanced pro-inflammatory processes influencing retinal capillary function.<sup>93,94</sup> Anti-VEGF therapy shows some potential for the treatment of diabetic patients with macular oedema.<sup>95</sup>

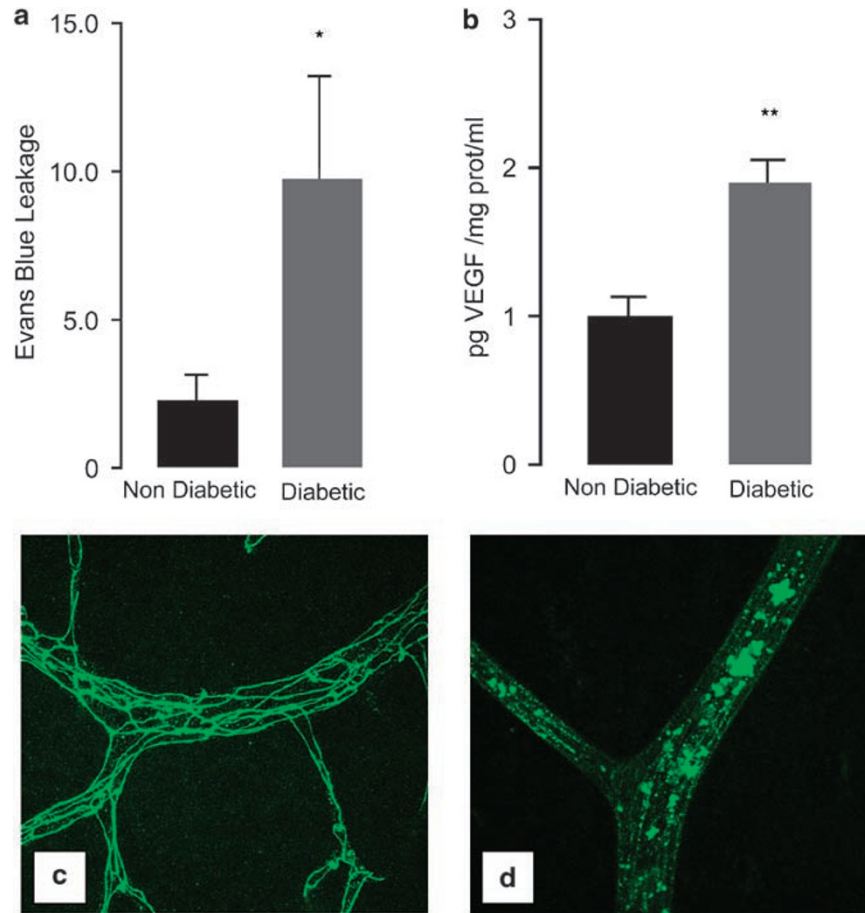
Abnormal retinal blood flow may contribute directly to the development of diabetic maculopathy (Figure 3). The hydrostatic pressure in the retinal capillaries is increased during long-term diabetes due to retinal arteriolar vasodilatation and increased retinal blood flow. Indeed, dilatation of the retinal arterioles has been shown to occur before the development of diabetic macular oedema<sup>96</sup> whereas reversal of vasopermeability and oedema following pan-retinal laser photocoagulation is associated with retinal vasoconstriction.<sup>64</sup> Supporting evidence for involvement of abnormal retinal blood flow in oedema comes from studies that indicate diffuse macular swelling is more than three times greater in diabetic patients with hypertension<sup>97</sup> and that reducing blood pressure protects against this end point.<sup>4</sup>

### Preretinal neovascularization

The proliferative diabetic retinopathy stage involves the formation of new blood vessels that develop from the venous side of the retinal circulation and penetrate the inner limiting membrane into the vitreous. Typically these new blood vessels are fragile and leaky and if left untreated can become enveloped by a thick and densely fibrous connective tissue layer. This fibrous tissue contributes to the formation of firm adhesions at the posterior hyaloid membrane and may eventually contract as it matures. Such traction can result in preretinal or vitreous haemorrhages or tractional retinal detachment leading to sudden visual loss. Proliferative retinopathy occurs in approximately 50% of patients with type I diabetes and in about 15% of patients with type II diabetes who have the disease for 25 years.<sup>98,99</sup>

The proliferative stage of diabetic retinopathy is driven by progressive retinal ischaemia that promotes expression of hypoxia-linked angiogenic agents. An array of hypoxia-regulated cytokines and growth factors has been implicated in the pathogenesis of retinal neovascularization. Of these vasoactive agents, VEGF has





**Figure 7** Blood retinal–barrier function in diabetes. Breakdown of the iBRB was assayed by leakage of Evans blue dye into the neural retina (a). After only 2 weeks of experimental diabetes in mice, there is an ~4-fold increase in vasopermeability compared to non-diabetic controls ( $*P < 0.005$ ). Quantification of VEGF release into the vitreous reveals that diabetes induces a significant increase in this vasoactive peptide (b) ( $**P < 0.01$ ). Diabetes also has a profound effect on the integrity of tight junctions (TJs) as shown by the change in staining pattern of the junctional complex component occludin in retinal flatmounts. Immunostaining for occludin-1 demonstrates integrity of the TJs between arterial, capillary, and venous endothelium in non-diabetic mice (c). Two weeks of diabetes significantly alters this configuration, with less defined occludin immunoreactivity occurring in the endothelial cell cytoplasm rather than at the plasma membrane (d).

received most attention and it is markedly elevated in the vitreous of patients with active proliferative retinopathy compared to those with quiescent proliferative retinopathy.<sup>100</sup> Indeed, delivery of exogenous VEGF peptide into the vitreous of normal primate eyes can itself stimulate neovascularization.<sup>101</sup> This and a wealth of related experimental evidence have rendered VEGF as a promising therapeutic target for proliferative diabetic retinopathy.<sup>102</sup> Although the use of VEGF-blocking agents has shown efficacy in patients with sight-threatening proliferative retinopathy, the use of such agents is controversial as they do not address the underlying vascular insufficiency and there are appropriate concerns that such therapy could compromise retinal neuroglial and functional microvascular survival.<sup>103–105</sup> There is a pressing need for

phase III clinical trials of anti-VEGF strategies in the context of diabetic retinopathy.

### Summary

Diabetic retinopathy is a multi-factorial condition arising from the complex interplay between biochemical and metabolic abnormalities occurring in all cells of the retina. Identification of a precise pathogenesis that links the progressive neuroglial and microvascular damage occurring in the diabetic retina remains a valid but somewhat elusive goal. In the current review, a unifying haemodynamic framework has been proposed that not only explains how the biphasic changes in retinal blood flow observed in diabetes may be interrelated, but also how these changes may contribute directly to the

development of diabetes-related microangiopathy and vision loss (Figure 3). It is hoped that this proposal encourages future studies that seek to improve our understanding of retinal haemodynamics and its role in the pathogenesis of diabetic retinopathy. Such research would be timely, because development of new treatments for this disease has been hampered by the slow time course over which it develops. If it can be proven that haemodynamic changes are prognostic indicators for diabetic retinopathy progression, robust measures of retinal blood flow change in patients could become an important surrogate end point for clinical drug trials that may ultimately improve the prognosis for patients with this complication.

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