Diabetic retinopathy: current and future methods for early screening from a retinal hemodynamic and geometric approach

Georgios Leontidis*, Bashir Al-Diri*, Andrew Hunter*

*University of Lincoln, School of Computer Science
Brayford pool, LN67TS, Lincoln, United Kingdom
Correspondence Author: gleontidis@lincoln.ac.uk
Tel: +44(0)1522886873

Abstract

Diabetic Retinopathy (DR) is a major disease and is the first cause of blindness in United Kingdom. Only in England 4200 new cases appear every year and 1280 lead to blindness. DR is a result of diabetes mellitus disease, which affects the retina of the eye and specifically the vessel structure. The elevated levels of glucose cause a malfunction in the cell structure, which affect the vessel wall and in severe conditions lead to them break. Quite a lot of research has been carried out for detecting the different stages of DR, but very few and versatile research has been done for the detection of DR early before the appearance of any lesions. In this review we approach the topic from the functional side of the human eye and how hemodynamic factors, which are impaired by diabetes, affect the vascular structure.

Keywords: Diabetes mellitus, Diabetic retinopathy, Diagnosis, Geometric features, Hemodynamic, Microcirculation, Retina

A critical approach and an effort to summarize the previous studies, the methods that were used and the limitations that were adopted, are intended in this manuscript. Diverse and comprehensive research is being conducted in this field, both from clinicians and computer scientists that creates the need of a deep understanding of the mechanisms underneath the development of DR. This fact enhances the necessity for a comprehensive approach in the functional impairment and the retinal vasculature alterations during the development and progress of diabetes, until the first lesions appear in the retina.

Since DR could be prevented with early diagnosis, it is useful to focus on finding and categorizing all these changes that are triggered by the progress of diabetes. In addition to the review of the past and current research, in this article will be attempted to approach possible future trends and describe some challenges that arise from the study of the changes in retina vessels during the progress of diabetes as well as how these changes are depicted in a color fundus image.

Anatomy of the retina

The vertebrate retina is composed by ten distinct layers, which are categorized from closest to farthest from the vitreous body [1]. The retina of the eye is a light-sensitive layer of tissue, which is found at the inner surface of the eye and enables the conversion of the incoming light into a neural signal so as to be further processed in the visual cortex of the brain. It remains the best-studied part of the human brain. It is easily accessible nowadays with many different methods (ophthalmoscope, fluorescein angiogram, OCT etc.) both from scientists and clinicians. Its importance is highlighted from the fact that, according to estimations, around 80% of all sensory information in humans originates from retina [2, 6-8].

As described above, retina is a layered structure of neurons interconnected with synapses from which only the photoreceptors

cells are light sensitive. There are two main types of photoreceptors: a) rods, which are responsible for black-and-white vision, mainly in dark light, and b) cones which make the eye perceive colour and support daytime vision. A third one is also found which is a much more rare type of photoreceptor, the intrinsically photosensitive ganglion cell, which responds to light in the absence of all rod and cone photoreceptors.

In humans the entire retina is approximately 24mm in diameter [8]. It contains 7 million cones and 75 to 150 million rods. An important part of the retina is the optic disc, which is often called the blind spot, since it has no photoreceptors. It is found at the optic papilla, a nasal zone where the optic-nerve fibres leave the eye [4-6]. In the fundus image, figure 1, this can be found as the white oval area, extending in an area of $3mm^2$. Temporal to the optic disc we find the macula, a small and highly sensitive part of the retina responsible for detailed central vision. In the very centre of the macula we meet fovea, whose main function contain sharp central vision, necessary in humans for activities that require visual details.

In section, the retina size is approximately 0.25mm in thickness, which varies with age [9]. It has three layers of nerve cells and two of synapses including the ribbon synapses. The connection between brain and retina is made via optic nerve, which carries the ganglion cell axons in the brain, and the blood vessels that are spread inside the retina [8].

Diabetic retinopathy

Although DR is a very common complication of diabetes still many cases are observed at a late stage where visual acuity is impaired and irreversible damage has already occurred [10, 11].

The prevalence of DR is found to increase with duration of diabetes. According to some very interesting results from the Australian Diabetes, Obesity and Lifestyle study in diabetic patients with less than 5 years in diabetic state, the prevalence of DR is less than 10%, but this number becomes more than 50% in those patients having diabetes for 20 or more years [12, 13].

The main risk factors of DR remain hypertension and hyperglycaemia but the hyperlipidaemia should not be excluded as well [14]. A number of epidemiological studies have highlighted the importance of hyperglycaemia in DR in addition to two pivotal studies: a) UK- prospective Diabetes study in patients with type 2 diabetes [15] and b) the Diabetes Control and Complications Trial in patients with type 1 diabetes [16, 17]. At the UK-PDS study there is another interesting part, which shows that by controlling the blood pressure level, reduces the risk of retinopathy regardless of the glycaemic level [18].

An important thing to keep in mind is that the isolated retinopathy signs (micro-aneurysms, haemorrhages and cotton wool spots) are found to be more common now in subjects without diabetes and hypertension, in comparison with what had been previously believed [13].

At an early stage DR affects the endothelial cells and the structure of muscle cells and leads to the loss of pericytes [19]. The proliferation of the endothelial cells and the thickening of the membrane cause vascular occlusion, whereas pericyte loss is responsible for the formation of micro-aneurysms. The big challenge remains to detect and understand the microvascular hemodynamic abnormalities in a stage that there are no morphological alterations in the retina. In this stage the clinicians could be able to intervene and prevent the progress of the disease still in a reversible stage.

Unfortunately the pathogenesis of DR is not yet fully understood both in cellular and molecular level limiting the options for effective therapeutic interventions early, while the disease still develops [20, 21]. It remains to be fully understood the exact mechanism that triggers the formation of the microvascular lesions.

In general, hyperglycaemia appears to be sufficient to initiate the development of DR as revealed by some experiments in animals, which were made hyperglycaemic in the lab

[22-24]. At the same time similar studies have shown that by intensively and sufficiently controlling hyperglycaemia leads to the inhibition of the development of DR [25].

Possible geometric alterations in the retina might indicate the existence of a systemic disease. Functional changes can be depicted in the retina vasculature and also be measured using devices like flow velocity meters, oximeters etc. Assessing these functional and hemodynamic changes in a qualitative way might help to bypass the limitations of studying only the morphological features. Moreover it can help differentiate between diseases that their structural effect in the retina is ambiguous and prone to misinterpretation.

Blood Supply

The retinal circulation is directly observable and has its own blood supply, which comes from behind the eye and enters the retina through the optic nerve. It is an end arterial system without anastomoses. The central retinal artery brings the blood into the retina and the central retinal vein drains the blood out of the eye leading the blood back to the heart for re-oxygenation [26, 27].

Screening of the retina

Retina remains the only non-invasive way of viewing human vessels. Using proper techniques, retina is visible from the outside, which assists on the imaging of the retina and brain tissue non-invasively [28, 29]. Moreover, due to the fact that retina is a highly metabolically active tissue with a double blood supply, it allows direct non-invasive observation of the circulation [30, 31].

The reason why retina is so much studied remains the fact that both eye and other diseases that affect the circulation and the brain can be illustrated in the retina [32, 33]. Macular degeneration and glaucoma are among the most prominent diseases and with DR the most important causes of blindness in the developed world [34].

In addition to the eye diseases, a number of systemic diseases also affect the retina. Complications of such diseases include diabetic retinopathy from diabetes mellitus, hypertension from cardiovascular disease and multiple sclerosis [35].

During the last decades, the advances in the non-invasive techniques that are used for measuring several features in the retina have led to the exploration of different aspects on the retinal hemodynamic, geometric features and blood flow regulation both in normal and diseased human eye. Some examples of these techniques are the following: The retinal vessel analyser (RVA) [36], dye dilution technique for arterio-venous passage time (AVP) [37], blue field simulation (BFS) for the velocity and the number of leukocytes [38], bidirectional laser Doppler velocimetry (LDV) for the red blood cell velocity [39], laser Doppler flowmetry for the blood flow in the tissue of the optic disc [40] and colour Doppler imaging for the central retinal arterial blood velocity [41]. Regarding the measurement of retinal blood flow another methodology includes the video fluorescein angiography, which relies on the rapid injection of a small bolus fluorescein dye and the recording of the retinal images with a scanning laser ophthalmoscope in order to distinguish the resultant vascular fluorescence from the passage of fluorescent dye through the retinal circulation [42].

Blood flow mechanisms in diabetes

An impairment in retinal blood flow is one of the earliest abnormalities occurred in the human body in diabetes [43]. Only 4% of the blood flow delivered to the eye is distributed to the retina [44]. Impairment of retinal circulation results in blood flow alterations, which consequently affect the delivery of oxygen and metabolic substrates to the tissue. The maintenance of the function and structure of the retina is highly affected from these abnormalities [45].

Grunwald et al. defined the problem of the importance or not of the blood flow especially in comparison with perfusion pressure [46]. In their initial study in diabetic patients they found that both the mean blood pressure and perfusion pressure in all of their patients who were normotensive, were

significantly higher in those five patients whose blood flow did not decrease after 5 days and whose retinopathy deteriorated at 6 months. Another observation shows that there is evidence that increased blood pressure is correlated with increased prevalence of retinopathy [47-49]. In the Wisconsin study was found that systolic blood pressure is a significant predictor of the incidence of diabetic retinopathy, while diastolic blood pressure can be an important predictor of the progression of the disease [48].

Perfusion pressure can also be of importance in diabetic retinopathy early screening, since the normal auto-regulatory response mechanism of vessels is impaired. Perfusion pressure can be calculated by subtracting the intraocular pressure from the two-thirds of the mean arterial pressure [50].

In another study using light stimulation in patients with well controlled type 1 diabetes with no signs of retinopathy showed that the functional abnormalities consist of reduced or no dilation of retina vessels as well as reduced or no constriction of retinal arteries as a response to the increase of intraluminal pressure [51, 52]. Very interesting finding [53, 54] was that flickering light stimulates and activates auto-regulatory mechanisms that dilate the retinal blood vessels and affect the blood flow by enhancing the circulation. In healthy subjects the response relates to the increase of the retina vessels by 2-4% whereas in diabetic patients there is slight or no response to the stimuli at all. An observation of the response of the retina vessels to increased intraluminal pressure and how the vascular mechanisms manage to regulate this, suggests that in normal arteries, an elevation in intraluminal pressure leads to the constriction of the vessels or to the dilation under pressure reduction. All of these occur by using the inherent mechanisms in the vascular smooth muscles that are independent of any hormonal, neural or metabolic influences. This mechanism is termed myogenic response [55].

It can be inferred from the above studies, that blood flow is an important window for understanding and measuring the changes during the progress of the disease. Every single change either it is in the vessel structure or in the microcirculation directly affects the blood flow inside the vessels. It is a fact that most of the studies use different methods to take such measurements and different metric system. It still remains a very difficult task to associate changes in blood flow with the progress of a disease since many other factors might influence the blood flow, which have to be excluded in the first place.

Biomarkers

A few tests were run to evaluate the response of the retina vessel structure to different stimuli, measuring at the same time these changes in vascular diameter either with laser Doppler flowmetry or with the dynamic retina vessel analyser [55-58]. Besides the measurements that were taken in different segments, some retinal vascular calibre changes were identified and associated with early consequences of diabetes making them candidates to become biomarkers of risk for diabetic complications.

A biomarker can be defined as a feature that is accurately and objectively measurable and is evaluated as an indicator of regular biological and pathogenic processes or responses in a specific drug treatment [59]. It is clear that some systemic markers like blood pressure, duration of diabetes, glucose level and lipid levels are definitely relevant factors, but on the other hand they cannot be used to identify the proliferation of DR [60]. It is observed that even patients under good glycaemic control can be worsen rapidly, in contrast to patients with poor control that might remain in stable condition [61]. This led to the identification of different phenotypes of progression taking into account the characteristics of the retinal lesions.

An important thing in defining the rudimental value of a novel biomarker [62] is the statistical evaluation with common statistical methods like area under the curve (AUC) or C-statistic but also with novel methods such as weighted net reclassification index (WNRI) and/or net benefit (NB). Using standard statistics we can only obtain information for the overall improvement,

differentiating over the whole range of possible decision thresholds. On the contrary, using more advanced techniques for decisions or analytic performance measurements like WNRI or NB we can obtain clinically useful results using a smaller range of thresholds with tested relevance in medical applications [49].Regarding diabetic retinopathy, until this review, there is no biomarker that satisfies the above criteria although research is conducted in several areas.

Time of testing plays a crucial role in accurately and reliably measuring the hemodynamic features at fixed state. Diverse indications of the abnormalities in diabetic patients are observed, which are assumed to be attributed to the influence of blood glucose level during the testing [63, 64]. Applying some stimuli in the retina can cause abnormal auto-regulation of blood flow in diabetic retinopathy patients, with a simultaneous increase of the flow, in parallel with the proliferation of DR [65].

Changes in the retinal architecture may result in impaired space filling and microcirculatory transport non-uniform shear distribution in branches and bifurcations. Moreover it might cause reduced energy efficiency in blood flow giving a strong indication of early disease state [62].

Pathology in diabetic retina

The pathological processes during diabetes are initially subtle but affect the whole hemodynamic functionality of the retina. During normal state, the auto-regulation mechanisms keep the blood flow constant in all the range of systemic blood pressure and intraocular pressure [3, 66-68]. The vessel responses are regulated locally by targeting the smooth muscle cells in arterioles and capillary pericytes [68-70]. On the contrary, during diabetes, there are changes in local vasoactive factors and the response of pericytes to these factors is altered as well [71- 73].

Tight junction complex proteins help in the creation of the blood-retinal barrier. In order to maintain normal neural function, the tight junction is responsible for the connection of the endothelial cells in the brain and retina [74]. In some diseases like DR, the actions of the vascular endothelial growth factor (VEGF) and cytokines on the tight junction proteins affect the vascular permeability and cause changes in the blood retinal barrier. The importance of blood-retinal barrier (BRB) can be highlighted from the fact that it is responsible for the prevention of certain substance entering into the tissues of the retina. BRB is formed from tight-junctions between retinal epithelial cells and non-fenestrated capillaries of the retinal circulation.

According to emerging evidence, neurodegeneration has been found to occur early in the pathogenesis of DR. In addition to neural apoptosis some changes in glial cells (non-neurons) occur as well and the process is known as reactive gliosis. It is still unclear which of the two processes occurs first in the degeneration process. The most important mechanisms that mediate the neurodegeneration process are a) oxidative stress, b) extracellular glutamate accumulation and c) reduction of neuro-protective factors synthetized by the retina [75].

The term oxidative stress, as defined, describes the imbalance that occurs between the ROS and the antioxidant defenses of a living system [76]. Tissue damage and pathophysiology is triggered by oxidants like ROS and reactive nitrogen species (RNS). The oxidative stress, which is caused by hyperglycemia, is considered an important pathway of diabetic microvascular complications [77]. There is strong evidence that the correlation between hyperglycemia, redox homeostasis and oxidative stress is responsible for the pathogenesis of DR [78, 79]. Another hypothesis supported that the oxidative species are responsible for the development and progression of DR [80]. Animal studies showed that oxidative species contribute to the resistance of retinopathy since they prevent proliferation after the good glycaemic control has been established [81]. *Brownlee* suggested that oxidative stress is very important since it links all the damaging biochemical pathways induced by hyperglycaemia in DR [82].

The Hoorn study reported the significance of subclinical inflammation to the development of DR [83]. DR is considered a low-grade inflammatory disease affecting the leukocytes' rolling and adhesion [84]. Nowadays the role of inflammation has been enhanced and it is considered very important though complex and unclear. Inflammation is triggered by factors such as hyperglycaemia, oxidative stress, hypertension etc. but this creates a chain reaction since inflammation propagates these pathways further through cytokines, VEGF signalling, adhesion molecules, enhanced RAGE expression, Nitric oxide regulation and NF-kβ signaling. The subclinical inflammation via e-NOS leads to increased intraocular pressure [85].

Diabetes effect in vessel structure

Hyperglycaemia leads to intramural pericyte death and thickening of the basement membrane, which contributes highly to the alterations in the integrity of the retinal blood vessels. This fact causes changes to the blood-retinal barrier and vascular permeability [86, 87].

During the hyperglycaemic state the endothelial cells align and elongate in the direction of shear stress modifying some of their functions at the same time. Shear stress is defined as the component of stress coplanar with a material cross section [88]. The endothelial cells respond to the increased shear stress and produce more a vasodilator named nitric oxide (NO), which causes the expansion of the blood vessels. This homeostatic reaction of the vessels occurs in order to restore the normal shear stress by decreasing the blood flow velocity [89, 90].

Another major issue is the development of atherosclerotic plaque, the hardening and thickening of arterial wall, due to the reactive oxygen species and inflammation. These two factors can be supressed by the nitric oxide. If the endothelial cells do not produce enough nitric oxide in response to shear stress in a diabetic state, can contribute to the development of atherosclerosis to diabetic patients [91].

The pulsatile flow of the blood through the vessels activates the endothelial nitric oxide (ENO) [92]. The shear stress, which is caused from the blood pressure in every beat of the heart, makes the vessels stretch and relax since the column of blood inside the vessels has not steady flow. The role of the ENO is to maintain the diameter of the blood vessels so as to preserve the perfusion of tissues at optimal levels. Vascular endothelial growth factor mediates the release of NO from human umbilical venous endothelial cells [93, 94].

High sugar levels cause alterations to the structure of the proteins. The endothelial cells attach to the proteins that are placed underneath them. The collagen, which is important substance for the elasticity of the vessel wall, becomes glycated in the high blood sugar state [95]. This means that sugar attaches to one of the collagen amino acids and creates a chain reaction. When a cell attaches to a glycated collagen there is a change in how it responds to the blood flow. For instance, the cells do not align in the flow direction and they do not even release nitric oxide. The production of advanced glycation end products is increased by the increase of superoxide anion [91].

Metabolic abnormalities are a main characteristic of diabetes, which include hyperglycaemia, free fatty acids and insulin resistance. These three factors provoke molecular mechanisms, which in their turn alter the function and the structure of the vessels. Oxidative stress is one of those affected, in addition to the malfunction of the intracellular signal transduction [91, 96, 97].

One very important concept defines that hyperglycaemic-induced oxidative stress, very common in diabetic retinopathy, mediates endothelial malfunction in diabetic patients. This is proven by the observations that intra-arterial infusion of ascorbic acid restores endothelium-dependent vasodilation in healthy subjects exposed to a hyperglycaemic clamp as well as in patients with type 1 and type 2 diabetes [91, 98].

Vascular muscle cell apoptosis in atherosclerotic lesions is also increased, in

such a way that patients with diabetes are prone to having fewer smooth muscle cells in the lesions, which increases the tendency for plaque rupture. The signalling pathways in the cells are affected totally from the way that cells adhere to the substrate proteins. Moreover since the cells attach to the glycated collagen in a completely different way than normal collagen, leads to different way of responding to the mechanical forces. Although the high blood sugar state is more frequent, the cells response to blood flow is altered even in low blood sugar levels [91].

The arterial wall is becoming stiffer with the aging but diseases like diabetes can accelerate this natural process. The arterial muscle cells contraction and relaxation are affected by the impaired endothelial cells which affect the wall stiffness by the modification of the isometric tone [99].

Hemodynamic changes in diabetes and DR – Experimental results in literature

Burgansky et al. studied the effect of diabetic retinopathy to the blood flow velocity in 42 diabetic patients and 38 healthy subjects finding that the velocity was slower in DR patients than healthy subjects (3.74±1.09 for DR and 4.19±0.99 for healthy – p-value <0.001) [100, 101].

Moreover they investigated the early hemodynamic alterations in patients with diabetes before the first lesions appear to the retina. In this study the blood flow velocity in the retinal vasculature of adult-onset diabetic patients with no signs of diabetic retinopathy (23 eyes) was compared with that of age-matched healthy subjects (51 eyes). For all the participants' measurements of blood glucose level, glycosylated haemoglobin (HbA1C), body mass index, intraocular pressure, systemic blood pressure and heart rate were taken. According to their results, the blood flow velocity in arteries was 4.7±1.7 mm/sec in the diabetic group, which is significantly higher than that of the healthy group 4.1±0.9mm/sec, with p-value=0.03. The velocity in venous in both groups was slower than arteries (3.8±1.2 mm/sec in DM group and 2.9 mm/sec in healthy group with p-value<0.0001). It is worth mentioning that in the diabetic group, the velocity values in both arteries and veins were not correlated to the duration of diabetes, the level of glucose, HbA1C or BMI [68, 101].

It is apparent that the increased velocity in diabetic patients compared to healthy subjects is opposed to the findings in DR patients [88]. That means that the relationship between the patient and healthy blood flow velocity reverses during the development of morphological alterations in the retina, as arteries reach their limits and capillary resistance defines the flow volume. In two studies [102,103] was found that blood flow velocity decreases over time in some but not all diabetic patients.

Takahiko et al. assessed the blood flow in the bilateral central arteries in 50 insulin-dependent diabetic patients without any signs of DR and they used 20 sex-and-age matched normal subjects as a comparison. For the measurements they used Duplex Doppler sonography [104]. The parameters that they measured were peak-systolic velocity (PSV), end-diastolic velocity (EDV), time averaged velocity (TAV), resistance index (RI) and pulsatility index (PI). As was expected the results were different between diabetic patients and normal subjects. PSV, EDV and TAV were lower in diabetic patients (9.9±1.9, 3.2±0.9 and 5.1±1.1 cm/s respectively) than in normal subjects (11.1±1.4, 3.9±0.7 and 6.0±1.0 cm/s respectively) with p-value<0.05, p-value<0.01 and p-value<0.01 respectively. Regarding the RI index it appeared to be higher in diabetic patients (0.70±0.05) than in normal subjects (0.65±0.05) with p-value<0.01. Finally for PI there was no significant difference between diabetic patients and normal subjects (1.37±0.45 vs. 1.22±0.30). Another interesting finding is that RI is correlated with the levels of glucose (r=0.310, p-value=0.0248) but not with haemoglobin levels (r=0.184, p-value=0.202), blood pressure or duration of diabetes. The outcome shows that retinal artery blood flow velocities were decreased whereas vascular resistance was increased in diabetic patients without clinical signs of DR.

Grunwald et al. recruited 19 diabetic patients with less than 4 years of diabetes and 16 age-matched normal subjects. In their experiment they measured different venous segments according to the quality of the images with a mean value of 4.4 and 4.5 in diabetic patients and normal subjects respectively. Haemodynamic parameters like blood glucose, mean blood pressure, intraocular pressure, perfusion pressure, haemoglobin and duration of diabetes were taken into account. The total measured blood flow rate in the diabetic patients was 43.3(standard deviation (SD) 8.9) μl/min, which is significantly higher than normal subjects (38.5(SD 4.7) μl/min) by about 12% (p-value<0.05) [105]. A positive correlation was also observed between blood flow and disease duration.

The total venous cross-section was 93.5(SD 20.3) cm^2x10^{-5} in diabetic patients, much higher than that of normal subjects (83.6(SD14) cm^2x10^{-5}) by about 12% with p-value<0.05. Again a correlation was observed between the total venous cross-section and duration of diabetes (r=0.34, p-value<0.05). Regarding the blood flow velocity in the largest retinal vein in both eyes appeared not to be significantly different between normal subjects and diabetic patients (1.79(SD 0.22) cm/s and 1.72(SD 0.39) cm/s respectively) and no significant correlation was observed between the velocity and duration of the disease(r=0.05, p-value<0.1). A very interesting part of this experiment was the measurement of the retinal vascular regulatory response in hypoxia which was defined as the

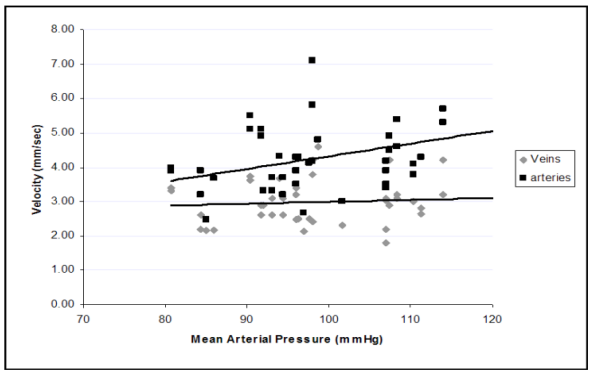

Figure 1. Blood flow velocity correlation with the mean arterial pressure using Retinal Function Imager [68].

percentage decrease in blood flow rate, blood flow velocity and large venous diameter between normal room air breathing and 100% oxygen breathing provided externally. This magnitude was found to be -11.6%(SD 4.5%) for venous diameter, -35.2%(SD 8.4%) in blood flow velocity and -49.2%(SD 7.8%) in blood flow rate but showing no significant difference from those of the normal subjects (-12.6%(SD 4.1%), -38.2(SD 10%) and -53%(SD 8.8%) respectively). Finally they found no significant correlations between any of the hemodynamic variables measured.

In another study [106], 12 normal subjects and 18 diabetic patients with background retinopathy were used for taking measurements of the total retinal volumetric blood flow and venous diameter in a similar way as the above mentioned experiment, using the same hemodynamic parameters. They found a positive linear correlation between the venous diameter measurements and the maximum erythrocyte velocity using a bidirectional laser Doppler in four to five major retinal veins both in normal subjects and diabetic patients. The results showed a significantly larger blood flow and venous cross section in the diabetic patients than normal subjects (p-value=0.02 and p-value=0.001 respectively). Blood flow was found significantly larger in temporal retina than in nasal retina in normal subjects (p-value=0.0008) and diabetic patients (p-value=0.0002). The measured blood flow was also significantly different between the superior and inferior retina in diabetic patients (p-value=0.03) but not in normal subjects.

Bursell et al. conducted a comprehensive experiment investigating the retinal blood flow changes in patients with type 1 diabetes and age-matched normal subjects [107]. It was also investigated whether blood glucose levels can alter the retinal blood flow and whether this can influence blood flow data in cross-sectional studies. Fluorescein angiography was used and blood glucose levels were adjusted in 3 levels using a glucose clamp in order to achieve values at 100, 200 and 300 mg/dl, taking blood flow measurement in each of these levels. Retinal blood flow was found to

be significantly decreased in diabetic patients (19.4±4.6 arbitrary units, p-value<0.01) in comparison with the blood flow in normal subjects (28.7±6.4 arbitrary units). During the glucose clamp in diabetic patients the retinal blood flow increased at the 200 mg/dl and to 300 mg/dl (21.5±4.7 arbitrary units, p-value<0.05 and 25.9±8.8 arbitrary units, p-value<0.01 respectively) in comparison with the 100mg/dl level (16.3±3.8 arbitrary units). In addition to the blood flow they took measurements of arterial and venous diameters but the results presented no significant differences in all the glucose clamps and between the two groups.

The rate of retinal blood flow depends on several factors, which determine the perfusion pressure and the vascular resistance. Perfusion pressure is the pressure that drives the blood into the retinal vasculature. Vascular resistance is generated by the combination of the retinal vessels and the blood viscosity [63].

Blood flow rate (Q) using the LDV method has been found to vary with $D^{2.76\pm0.16}$ in the arteries (values of diameter (D) between 39 and 145μm) and $D^{2.84\pm0.12}$ for the veins (values of D between 64 and 177μm) [108]. These values are in close agreement with Murray's law [109], which calculates an exponent value of 3 for a vascular system that seeks an optimum compromise between blood volume and vascular resistance or in other words that minimizes its resistance for a given volume. Feke et al found an exponent of 4.1 for D>100μm [110] and Garcia et al a 3.35 for D between 84-177μm [111]. In another study

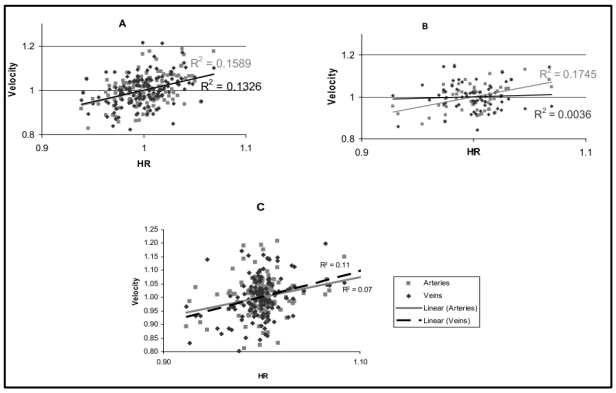

Figure 2. Retinal blood flow-velocity and heart rate correlation of all the data used. A. Healthy group, B. Diabetes group, C. DR group [68].

using dye delivery technique found an exponent of 2.9 for retinal arterioles and arteries with a D between 20 and 80μm [112].

In literature, as far as normal subjects are concerned, the values of the blood flow rate between studies varies between 30 and 38 μl/min [106, 108] and from 65-80 μl/min (102, 110, 111). These differences in measurements are attributed to the fact of different methodological approaches.

As outlined by *Burgansky*, the correlation of blood flow velocity to physiological parameters is very important in order to understand the effects that diabetes can have to the human body functionality. In figure 1 it is shown that for healthy group the flow velocity in arterioles is positively correlated with mean arterial pressure (r=0.29, p-value=0.006). For the systolic blood pressure the correlation coefficient r=0.3 the p-value=0.04 and for the diastolic blood pressure the values are for r=0.4 and p-value=0.009) [68].The interesting part in this is that there was no significant correlation observed between blood flow velocity and mean arterial pressure in both pre-retinopathy and diabetic retinopathy patients. This indication does not exclude the fact that there might be reduced correlation since it could be impaired by other factors while diabetes develops and progresses.

The average heart rate influence in the average velocity was also investigated but no statistically significant correlation was found in either healthy, diabetic or DR subjects. The relationship was assessed by correlating the heart rate and velocity measurements recorded simultaneously (figure 2).

In a very recent comprehensive study [66], type 1 diabetic patients aged 12-20 years were used including clinical assessment and retinal photography. A total number of 1.159 patients were recruited from which the 944 of them had gradable photographs and 170 of them had retinopathy. The following geometric features were evaluated: branching angles, arteriolar and venular tortuosity, optimality deviation and length-to-diameter ratio from digitized photographs. In addition, their efforts focused as well on the association of the geometric

characteristics with diabetes duration, A1C haemoglobin level and systolic blood pressure. An association between the older age and decreased arteriolar (p-value=0.024) and venular tortuosity (p-value=0.002) was found. Another interesting finding was that female subjects had larger arteriolar branching angle than male (p-value=0.03). Moreover longer duration of diabetes was associated with larger arteriolar branching angle (p-value<0.001) as well as with increased arteriolar optimality deviation (p-value=0.018). Increased level of A1C haemoglobin was associated with increased arterial tortuosity (>8.5% vs. <=8.5%, p-value=0.008). Length-to-diameter ration was associated with higher systolic blood pressure (p-value=0.002) and increased arteriolar length-to-diameter ration was associated with higher total cholesterol levels (p-value=0.044) and decreased venular optimality deviation (p-value=0.044). It is also mentioned that these associations remain even after controlling A1C, retinal vessel calibre and retinopathy status and was observed in non-retinopathy subjects as well.

Unfortunately, when it comes to review, compare and summarize such studies some problems arise that make the interpretation a bit problematic. Most of the studies do not use the same techniques for measurements and they do not have the same requirements for selecting participants. The results cannot be compared when the groups of participants have different demographic background and/or different medical record (other diseases etc.). On the other hand these results give us a good indication on what is happening in the retina from many different perspectives both from the hemodynamic and geometric side. This can help us move forward and plan future studies, which will be based on the previous findings, trying at the same time to increase the factors that are taken into account in order to enhance the robustness and reliability of the analysis and the results.

Oxygen perfusion

It is still uncertain and unclear the exact cause of the elevated oxygen saturation in DR but it is evident that normal response of the retinal circulation in preventing hyper-oxygenation is impaired by hyperglycaemia [65].

Oxygen distribution is another major factor in the vessel wall oxygen perfusion. The process of the oxygen distribution can be affected by some factors including capillary non-perfusion and shunting, thickening of capillary basement membranes and oxygen affinity of haemoglobin in diabetic patient [102].

If the capillary network is at some point shunted, the blood may bypass it through dilated channels. During this state some of the capillaries are closed and some dilated. Some studies using fluorescein angiography have shown that dilated capillaries force the blood to pass from arterioles to venules in the retina leading to capillary non-perfusion [113]. This “unhealthy” process makes the transport of blood faster than normal making the venules blood hyperoxic and the rest of the blood hypoxic in these non-perfused areas. The capillary non-perfusion in addition to shunting disturbs the normal blood flow altering the normal oxygenation that may lead to different pathologies. As a consequence the non-perfused areas do not extract the oxygen from the haemoglobin, so these venules have higher oxygenation levels making this tissue hypoxic and thus ischemic.

One of the observations during DR is that capillary walls thicken which can lower oxygen delivery levels. It is assumed that in this situation the oxygen is inhibited from the efficiently diffusing and perfusing vessels contributing probably to the elevated oxygenation of the blood. Knowing the high importance of the oxygen for the functionality and preservation of tissues it can be inferred that the mal-distribution during the disease state makes the tissue hypoxic, elevating the demands of oxygen thus increasing the blood flow to deliver more oxygen. The above way is one reason that total blood flow can be increased in DR [113].

It is easily understood that dead tissue cannot consume oxygen. Tissue degeneration lowers the total amount of oxygen extracted by blood vessels increasing simultaneously the

venous oxygen saturation as mentioned in [114].

It is speculated that the affected microcirculation in diabetes has an effect on retinal vessel saturation [115]. Pathogenesis in diabetic retinopathy has been linked to retinal hypoxia, which triggers neovascularization and retinal oedema [116].

Bahram et al. conducted a comprehensive experiment in order to determine the retinal oxygen saturation trend with onset of diabetes and increasing severity of DR by comparing diabetic groups with and without retinopathy [114]. For this purpose they used a fundus camera-based dual wavelength snapshot oximeter to take images of the retina for the whole recruited group and analyse them to determine oxygen saturation in the major arteries and veins. It was found that in normal subjects the saturation in arteries was 92.3±4.2% and in veins 57.2±6%, in diabetic patients without DR was in arteries 96.3±8.6% and in veins 58.7±7.5%, in diabetic patients with mild to moderate non proliferative DR in arteries it was 97.7±5.8% and in veins 61.1±7.6%. The saturation for diabetics with severe non-proliferative DR was in arteries 102±10.2% and in veins 66.8±8.4%. In patients with proliferative DR in arteries was 103.6±8.7% and in veins 66.6±10.2% and finally in all diabetics with DR combined in arteries was 100.4±7.6% and in veins 64.2±8.4%. From this study it is clear that there is a trend of increasing the retinal oxygen saturation from healthy subjects to non-diabetic retinopathy group and to diabetic retinopathy patients.

During DR the oxygen perfusion might be influenced by the impaired blood flow. In *Jorgensen et al.*, 156 diabetic patients, 48 with type 1 and 108 with type 2 diabetes were recruited, in addition to 80 age-matched normal subjects [117]. For the normal controls any other diseases like ocular diseases, diabetes or hypertension were excluded. They used a retinal oximetry device in order to measure the oxygen saturation in veins and arteries. As observed in proliferative DR patients the arterial saturation was significantly higher than normal subjects and in diabetic patients with retinopathy not requiring treatment, whereas there was no significant difference with diabetic patients without retinopathy.

Regarding the veins, the diabetic patients with or without retinopathy presented significantly higher saturation than normal subjects. Another important observation shows that the oxygen extraction decreases with increasing severity of retinopathy, namely from normal subjects until the last stage of proliferative DR.

Expert commenting

Unfortunately, despite the great deal of research that is being conducted for detecting and diagnosing DR in an early stage before the first lesions appear, still remains unclear how the biochemical alterations in the human body during the development and progress of diabetes affect the retinal vessel structure and the hemodynamic functionality. The exact mechanisms and the sequence of them that lead to the appearance of the first lesions, like micro-aneurysms or hemorrhages are still not very well understood. Because of that it is very difficult to evaluate the vasculature structure of a diabetic patient, monitor the changes that occur and then associate them with the progress and effect of diabetes. There is still no biomarker that can satisfy the criteria of reliability, accuracy and generalization with defined limitations, let alone when it comes to take into account some risk factors like age, gender, duration of diabetes, other diseases etc. A definite and robust connection between the hemodynamic features (blood flow volume rate, blood flow velocity, oxygen perfusion, circulation time, vessel wall stiffness, body functionality etc.) and the geometric features (vessel width, tortuosity index, wall thickness etc.) is needed. This would help us understand how a change in the hemodynamic functionality, which will only be correlated with diabetes, is depicted in the vasculature both qualitatively and quantitatively. Having such a direct connection with concrete features and specific conditions could give us a biomarker, reliable enough to use it as a predictor of early progression or inherent

development of DR before the first lesions appear.

Five-years view

As mentioned previously, quite a lot of ongoing studies investigate the possibility of early screening of DR. Even in the very recent literature, there is no definite and reliable connection of the changes in the retinal vasculature and the development of DR. Until recently, it was impossible to take oxygen saturation measurements in the vessel segments accurately and reliably. In the last years it has become possible to study the oxygen saturation in the retinal vasculature, taking accurate measurements and at the same time investigate whether oxygen perfusion impairment precedes or follows the development of DR. According to the wider trends, as well as the research activity in our research group, the next five years will be quite important in understanding the effect of diabetes in the retinal vasculature. Oxygen perfusion seems to play a crucial and quite distinctive role in all the stages of DR both in normal and diabetic subjects. It remains to have a direct correlation with some risk factors like age, gender, type of diabetes etc. and even more important to exclude any other diseases. This is crucial since it will validate that diabetes or DR only affects the measured features. A great challenge and another important observation is the auto-regulatory responses of the retinal vasculature to the altered hemodynamic function, which is yet unclear the way it is impaired in connection with the disease. Furthermore there might be some progress in understanding whether the final positions of the lesions in the vasculature are random or if they are determined by other factors and whether any changes to the vessels' position occur before the lesions finally appear in this specific area.

Financial and competing interest disclosure

This research was made possible by a Marie Curie grant from the European Commission in the framework of the REVAMMAD ITN (Initial Training Research network), Project number 316990. Apart from this, there is no other financial involvement or other affiliations with any organization or entity with a financial interest and there is no conflict with the subjects and topics is discussed in this manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized during the production of this manuscript.

Key issues

- Diabetic retinopathy is a major cause of blindness.
- There is no reliable biomarker yet that can predict or diagnose DR.
- Diabetes causes many changes to the body functionality that lead to DR.
- Oxygen saturation level in the veins and arteries is different between normal subjects, diabetic patients and DR patients.
- Endothelial cells' function is severely altered during diabetes, which is a major complication that can lead to DR.
- Blood flow and vessel structure is highly affected during the development and progress of diabetes.
- Risk factors like age, gender, duration of diabetes, other diseases, family history etc. are important parameters that have to be considered when studying the progress of diabetes.

References

Papers of special note have been highlighted as:
* of interest
** of considerable interest

1. Villegas GM. Electron microscopic study of the vertebrate retina.*The Journal of general physiology* 43, no. 6, 15-43(1960).
2. Hildebrand GD, Fielder AR. Anatomy and Physiology of the Retina. *Pediatric Retina*. 39-65 (2011)
   *** Great introductory source for the structure and physiology of the retina. It is a must-have book for understanding the functionality and the anatomy of the retina**
3. Riva CE, Sinclair SH, Grunwald JE. Autoregulation of retinal circulation in response to decrease of perfusion pressure. *Investigative Ophthalmology & Visual Science* , *21* (1), 34–38 (1981).
4. Navarro R. The Optical Design of the Human Eye: a Critical Review. *Journal of Optometry*. 2, vol. 2, Issue 1, 3-18 (2009).
5. Navarro R, Santamaría J, Bescós J. Accommodation-dependent model of the human eye with aspherics. *J. Opt. Soc. Am.* A 2, 1273-1280 (1985).
6. Oyster CW. The human eye. *Sinauer Associates*, (1999).
7. Dowling JE. The retina: an approachable part of the brain. *Harvard University Press*, (1987).
8. Cunningham, edited by Paul Riordan-Eva, Emmett T. *Vaughan & Asbury's General Ophthalmology*. (18th ed.). New York: McGraw-Hill Medical, (2011).
9. Alamouti B, Funk J. Retinal thickness decreases with age: an OCT study. *British journal of ophthalmology* 87, no. 7, 899-901 (2003).
10. Aiello LM. Perspectives on diabetic retinopathy.*American journal of ophthalmology* 136, no. 1, 122-135 (2003).
11. Ruta LM, Magliano DJ, Lemesurier R, Taylor HR, Zimmet PZ, Shaw JE. Prevalence of diabetic retinopathy in Type 2 diabetes in developing and developed countries. *Diabetic Medicine* 30, no. 4, 387-398 (2013).
    *** This review paper makes clear the importance of DR and the consequences that it has all around the world by making comparisons and summarizing according to risk factors and demographic data.**
12. Tapp RJ, Shaw JE, Harper CA et al. The prevalence of and factors associated with diabetic retinopathy in the Australian population." *Diabetes care* 26, no. 6 1731-1737 (2003).
13. Nguyen,TT., Wang JJ, Amirul Islam FM et al. Retinal Arteriolar Narrowing Predicts Incidence of Diabetes The Australian Diabetes, Obesity and Lifestyle (AusDiab) Study. *Diabetes* 57, no. 3, 536-539 (2008).
14. Nguyen TT, Wang JJ, Wong TY. Retinal Vascular Changes in Pre-Diabetes and Prehypertension New findings and their research and clinical implications. *Diabetes Care* 30, no. 10, 2708-2715 (2007).
15. Turner RC, Holman RR, Cull CA, Stratton IM, Matthews DR, Frighi V, Manley SE. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33)." *lancet* 352, no. 9131, 837-853 (1998).
16. Ohkubo Y, Kishikawa H, Araki E et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes research and clinical practice* 28, no. 2, 103-117 (1995).
17. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. Engl. J. Med., 329, 977–986 (1993).
18. UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ: British Medical Journal* 317, no. 7160 , 703 (1998).
19. Eva NH, Zhang AW, Mansour FA. Prevention of pericyte loss by trolox in diabetic rat retina.*Journal of Toxicology and Environmental Health Part A* 54, no. 6, 467-475 (1998).
20. Stitt AW, McGoldrick C, Rice-McCaldin A et al. Impaired retinal angiogenesis in diabetes role of advanced Glycation end products and Galectin-3. *Diabetes* 54, no. 3, 785-794 (2005).
21. Stitt AW, Curtis TM. Advanced glycation and retinal pathology during diabetes. *Pharmacological Reports* 57, 156 (2005).

22. Engerman RL, Kern TS. Experimental galactosemia produces diabetic-like retinopathy. *Diabetes* 33, no. 1, 97-100 (1984).
23. Kador PF, Akagi Y, Takahashi Y, Ikebe H, Wyman M, Kinoshita JH. Prevention of retinal vessel changes associated with diabetic retinopathy in galactose-fed dogs by aldose reductase inhibitors. *Archives of ophthalmology* 108, no. 9, 1301-1309 (1990).
24. Kern TS, Engerman RL. Capillary lesions develop in retina rather than cerebral cortex in diabetes and experimental galactosemia. *Archives of ophthalmology* 114, no. 3, 306-310 (1996).
25. Engerman RL, Kern TS. Aldose reductase inhibition fails to prevent retinopathy in diabetic and galactosemic dogs. *Diabetes* 42, no. 6, 820-825 (1993).
26. Funk RHW. Blood supply of the retina. *Ophthalmic research* 29, no. 5, 320-325 (1997).
27. Hayreh SS. Blood supply of the optic nerve head and its role in optic atrophy, glaucoma, and oedema of the optic disc. *The British journal of ophthalmology* 53, no. 11, 721 (1969).
28. Lupascu CA. Human Visual Perception and Retinal Diseases. *Proc. CREATE,* (2010).
29. Terai NM, Siegel HA, Stodtmeister R, Pillunat LE, Sandner D. Diameter of retinal vessels in patients with diabetic macular edema is not altered by intravitreal ranibizumab (lucentis). *Retina (Philadelphia, Pa.)* (2014).
30. Rice MJ, Sweat RH Jr, Rioux JM, Williams WT, Routt W. Non-invasive measurement of blood components using retinal imaging. USPatent 6477394 (2002).
31. Kim DY, Fingler J, Werner JS, Schwartz DM, Fraser SE, Zawadzki RJ. In vivo volumetric imaging of human retinal circulation with phase-variance optical coherence tomography. *Biomedical optics express* 2, no. 6, 1504-1513 (2011).
32. Wong TY, Klein R, Klein BEK, Tielsch JM, Hubbard L, Nieto FJ. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. *Survey of ophthalmology* 46, no. 1, 59-80 (2001).
33. Wong TY, Amirul Islam FM, Klein R et al. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA)." *Investigative ophthalmology & visual science* 47, no. 6, 2341-2350 (2006).
34. Williams RM, Baxter AH, Forrester J, Kennedy-Martin T, Girach A. Epidemiology of diabetic retinopathy and macular oedema: a systematic review. *Eye* 18, no. 10, 963-983 (2004).
35. Hayreh SS, Zimmerman B, McCarthy MJ, Podhajsky P. Systemic diseases associated with various types of retinal vein occlusion. *American journal of ophthalmology* 131, no. 1, 61-77 (2001).
36. Polak K, Dorner G, Kiss B et al. Evaluation of the Zeiss retinal vessel analyser. *British Journal of Ophthalmology* 84, no. 11 1285-1290 (2000).
37. Wolf S, Jung F, Kiesewetter H, Körber N, Reim M. Video fluorescein angiography: method and clinical application. *Graefe's archive for clinical and experimental ophthalmology* 227, no. 2 145-151 (1989).
38. Riva CE, Petrig B. Blue field entoptic phenomenon and blood velocity in the retinal capillaries. *JOSA* 70, no. 10, 1234-1238 (1980).
39. Sullivan PM, Davies GE, Caldwell G, Morris AC, Kohner EM. Retinal blood flow during hyperglycemia. A laser Doppler velocimetry study. *Investigative ophthalmology & visual science* 31, no. 10, 2041-2045 (1990).
40. Nilsson GE, Tenland T, Oberg PA. Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. *Biomedical Engineering, IEEE Transactions on* 10, 597-604 (1980).
41. Williamson TH, Baxter GM. Central retinal vein occlusion, an investigation by color Doppler imaging. Blood velocity characteristics and prediction of iris neovascularization. *Ophthalmology* 101, no. 8, 1362 (1994).
42. Wolf SF, Kiesewetter JH, Körber N, Reim M. Video fluorescein angiography: method and clinical application. *Graefe's archive for clinical and experimental ophthalmology* 227, no. 2, 145-151 (1989).
43. Cunha-Vaz, Jose G., Fonseca JR, José RF de Abreu, João JP Lima. Studies on retinal blood flow: II. Diabetic retinopathy. *Archives of ophthalmology* 96, no. 5, 809-811(1978).
44. Network, Choroidal Vascular. Developmental Anatomy of the Retinal and Choroidal Vasculature. *The Retina and Its Disorders* 179 (2011).

45. Clermont AC, Bursell SE. Retinal blood flow in diabetes. *Microcirculation* 14, no. 1, 49-61 (2007).
**** Very important and thorough paper that helps a lot in understanding the impairments in blood flow during diabetes in a more clinical approach**
46. Grunwald JE, Maguire AM, Dupont J. Retinal hemodynamics in retinitis pigmentosa. *American journal of ophthalmology* 122, no. 4, 502-508 (1996).
47. Knowler WC, Bennett PH, Ballintine EJ. Increased incidence of retinopathy in diabetics with elevated blood pressure: a six-year follow-up study in Pima Indians. *New England Journal of Medicine* 302, no. 12, 645-650 (1980).
48. Klein R, Moss SE, Klein BE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. XI. The incidence of macular edema. *Ophthalmology* 96, no. 10, 1501-1510 (1989).
49. Klein R, Klein BEK, Moss SE, Cruickshanks KJ. The Wisconsin epidemiologic study of diabetic retinopathy XV: the long-term incidence of macular edema. *Ophthalmology* 102, no. 1, 7-16 (1995).
50. Sinclair SH, Grunwald JE, Riva CE, Braunstein SN, Nichols CW, Schwartz SS. Retinal vascular autoregulation in diabetes mellitus. *Ophthalmology* 89, no. 7, 748-750 (1982).
51. Garhöfer G, Zawinka C, Resch H, Kothy P, Schmetterer L, Dorner GT. Reduced responseof retinal vessel diameters toflicker stimulation in patients with diabetes. *Br J Ophthalmol* 88, 887–891 (2004).
52. Mandecka A, Dawczynski J, BlumM, et al. Influence of flickering light on the retinal vessels in diabetic patients. *Diabetes Care*, 30, 3048–3052 (2007).
53. Hill MA, Meininger GA, Davis MJ, Laher I. Therapeutic potential of pharmacologically targeting arteriolar myogenic tone. *Trends Pharmacol Sci*, 30, 363–374 (2009).
54. Lorenzi M, Feke GT, Pitler L, Berisha F,Kolodjaschna J, McMeel JW. Defective myogenic response to posture change in retinal vessels of well-controlled type 1 diabetic patients with no retinopathy. *Invest Ophthalmol Vis Sci*, 51, 6770–6775 (2010).
55. Hill MA, Gerald A. Meininger GA, Davis MJ, Laher I. Therapeutic potential of pharmacologically targeting arteriolar myogenic tone. *Trends in pharmacological sciences* 30, no. 7, 363-374 (2009).
56. Garhöfer G, Zawinka C, Resch H, Huemer KH, Schmetterer L, Dorner GT. Response of retinal vessel diameters to flicker stimulation in patients with early open angle glaucoma. *Journal of glaucoma* 13, no. 4, 340-344 (2004).
57. Mandecka A, Dawczynski J, Blum M et al. Influence of flickering light on the retinal vessels in diabetic patients. *Diabetes care* 30, no. 12, 3048-3052 (2007).
58. Lorenzi M, Feke GT, Pitler L, Berisha F, Kolodjaschna J, McMeel JW. Defective myogenic response to posture change in retinal vessels of well-controlled type 1 diabetic patients with no retinopathy. *Investigative ophthalmology & visual science* 51, no. 12, 6770-6775 (2010).
59. Steyerberg EW, Pencina MJ, Lingsma HF,Kattan MW, Vickers AJ, Van Calster B. Assessing the incremental value of diagnosticand prognosticmarkers: a review and illustration. *Eur J Clin Invest*, 42, 216–228 (2012).
60. Hove MN, Kristensen JK, Lauritzen T, Bek T. The relationships between risk factors and the distribution of retinopathy lesions in type 2 diabetes. *Acta Ophthalmologica Scandinavica* 84, no. 5, 619-623 (2006).
61. Ribeiro ML, Nunes SG, Cunha-Vaz JG. Microaneurysm turnover at the macula predicts risk of development of clinically significant macular edema in persons with mild nonproliferative diabetic retinopathy. *Diabetes care* 36, no. 5, 1254-1259 (2013).
62. Kamran IM, Cheung CY, Lorenzi M, Klein R, Jones TLZ, Wong TY. Retinal vascular caliber as a biomarker for diabetes microvascular complications. *Diabetes care 36*, no. 3, 750-759 (2013).
**** Good establishment of the current research orientations in defining biomarkers for DR. It summarizes some current research approaches and the difficulties that arise in defining a biomarker.**
63. Pournaras CJ, Rungger-Brändle E, Riva CE, Hardarson SH, Stefansson E. Regulation of retinal blood flow in health and disease. *Progress in retinal and eye research* 27, no. 3, 284-330 (2008).
64. Ishii H, Jirousek MR, Koya D et al. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC β inhibitor. *Science* 272, no. 5262, 728-731 (1996).
65. Kohner EM, Patel V, Rassam SMB. Role of blood flow and impaired autoregulation in the pathogenesis of diabetic

retinopathy. *Diabetes* 44, no. 6, 603-607 (1995).
66. Sasongko MB, Wang JJ, Donaghue KC et al. Alterations in retinal microvascular geometry in young type 1 diabetes. *Diabetes Care* 33, no. 6, 1331-1336 (2010).
67. Robinson F, Riva CE, Grunwald JE, Petrig BL, Sinclair SH. Retinal blood flow autoregulation in response to an acute increase in blood pressure. *Investigative ophthalmology & visual science* 27, no. 5, 722-726 (1986).
68. Burgansky-Eliash Z. The Effect of Diabetes Mellitus on Retinal Function, *Diabetic Retinopathy*, source:InTech
69. Sims DE. The pericyte: a review. *Tissue and Cell* 18, no. 2, 153-174 (1986).
70. Shepro D, Morel NM. Pericyte physiology. *The FASEB Journal* 7, no. 11, 1031-1038 (1993).
71. Joussen AM, Poulaki V, Mitsiades N et al. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-α suppression. *The FASEB journal* 16, no. 3, 438-440 (2002).
72. King GL, Shiba T, Oliver J, Inoguchi T, Bursell SE. Cellular and molecular abnormalities in the vascular endothelium of diabetes mellitus. *Annual review of medicine* 45, no. 1, 179-188 (1994).
73. Bursell SE, Takagi C, Clermont AC et al. Specific retinal diacylglycerol and protein kinase C beta isoform modulation mimics abnormal retinal hemodynamics in diabetic rats. *Investigative ophthalmology & visual science* 38, no. 13, 2711-2720 (1997).
74. Harhaj NS, Antonetti DA. Regulation of tight junctions and loss of barrier function in pathophysiology. *The international journal of biochemistry & cell biology* 36, 7, 1206-1237 (2004).
75. Simó R, Hernández C. Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. *Trends in Endocrinology & Metabolism* 25, 1, 23-33 (2014).
76. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 40, no. 4, 405-412 (1991).
77. Cui Y, Kim DS, Park KC. Antioxidant effect of Inonotus obliquus. *Journal of Ethnopharmacology* 96, no. 1, 79-85 (2005).
78. Kowluru RA, Chan PS. Oxidative stress and diabetic retinopathy. *Experimental diabetes research* 2007; 43603, (2007) .
79. El-Remessy AB, Bartoli M, Platt DH, Fulton D, Caldwell RB. Oxidative stress inactivates VEGF survival signaling in retinal endothelial cells via PI 3-kinase tyrosine nitration. *Journal of Cell Science* 118, no. 1, 243-252 (2005).
80. Van den Enden MK, Nyengaard JR, Ostrow E, Burgan JH, Williamson JR. Elevated glucose levels increase retinal glycolysis and sorbitol pathway metabolism. Implications for diabetic retinopathy. *Investigative ophthalmology & visual science* 36, no. 8 1675-1685 (1995).
81. Kowluru RA, Koppolu P, Chakrabarti S, Chen S. Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. *Free radical research* 37, no. 11, 1169-1180 (2003).
82. Brownlee M. The pathobiology of diabetic complications a unifying mechanism. *Diabetes* 54, no. 6, 1615-1625 (2005).
83. Van Hecke MV, Dekker JM, Nijpels G et al. Inflammation and endothelial dysfunction are associated with retinopathy: the Hoorn Study. *Diabetologia* 48, no. 7, 1300-1306 (2005).
84. Miyamoto K, Ogura Y. Pathogenetic potential of leukocytes in diabetic retinopathy. *In Seminars in ophthalmology*, vol. 14, no. 4, 233-239 (1999).
85. Adamis AP. Is diabetic retinopathy an inflammatory disease?. *British Journal of Ophthalmology* 86, no. 4, 363-365 (2002).
86. Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN. Hyperglycemic crises in adult patients with diabetes. *Diabetes care* 32, no. 7 1335-1343 (2009).
87. Qaum T, Xu Q, Joussen AM et al. VEGF-initiated blood–retinal barrier breakdown in early diabetes. *Investigative ophthalmology & visual science* 42, no. 10, 2408-2413 (2001).
88. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 105, no. 9, 1135-1143 (2002).
89. Gross ER, LaDisa Jr JF, Weihrauch D et al. Reactive oxygen species modulate coronary wall shear stress and endothelial function during hyperglycemia. *American Journal of Physiology-Heart and Circulatory Physiology* 53, no. 5, H1552 (2003).
90. Beckman JA, Goldfine AB, Gordon MB, Creager MA. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. *Circulation* 103, no. 12, 1618-1623 (2001).
91. Creager MA, Lüscher TF, Cosentino F, JBeckman JA. Diabetes and vascular

disease pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation* 108, no. 12, 1527-1532 (2003).
92. Lu X, Kassab GS. Nitric oxide is significantly reduced in ex vivo porcine arteries during reverse flow because of increased superoxide production. *The Journal of physiology* 561, no. 2, 575-582 (2004).
93. Toda N, Imamura T, Okamura T. Alteration of nitric oxide-mediated blood flow regulation in diabetes mellitus. *Pharmacology & therapeutics* 127, no. 3, 189-209 (2010).
94. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: a clinical perspective. *Endocrine reviews* 22, no. 1, 36-52 (2001).
95. Wautier JL, Schmidt AM. Protein glycation a firm link to endothelial cell dysfunction. *Circulation Research* 95, no. 3, 233-238 (2004).
96. Hartnett ME, Stratton RD, Browne RW, Rosner BA, Lanham RJ, Armstrong D. Serum markers of oxidative stress and severity of diabetic retinopathy. *Diabetes Care*, *23*(2), 234-240 (2000).
97. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *Jama* 287, no. 19, 2570-2581 (2002).
98. O'Driscoll G, Green D, Rankin J, Stanton K, Taylor R. Improvement in endothelial function by angiotensin converting enzyme inhibition in insulin-dependent diabetes mellitus. *Journal of Clinical Investigation* 100, no. 3, 678 (1997).
99. Bank AJ, Wilson RF, Kubo SH et al. Direct effects of smooth muscle relaxation and contraction on in vivo human brachial artery elastic properties. *Circulation research* 77, no. 5, 1008-1016 (1995).
100. Burgansky-Eliash Z, Barak A, Barash H et al. Increased retinal blood flow velocity in patients with early diabetes mellitus. *Retina* 32, no. 1, 112-119 (2012). **** Very accurate and comprehensive description and measurements of the retina vessels blood flow in pre-retinopathy and post-retinopathy patients. The correlations with blood pressure and heart rate are very useful as well.**
101. Burgansky-Eliash Z, Nelson DA, Bar-Tal OP, Lowenstein A, Grinvald A, Barak A. Reduced retinal blood flow velocity in diabetic retinopathy. *Retina* 30, no. 5, 765-773 (2010).
102. Konno S, Feke GT, Yoshida A et al. Retinal blood flow changes in type I diabetes. A long-term follow-up study. *Investigative ophthalmology & visual science* 37, no. 6, 1140-1148 (1996).
103. Rimmer T, Fallon TJ, Kohner EM. Long-term follow-up of retinal blood flow in diabetes using the blue light entoptic phenomenon. *British journal of ophthalmology* 73, no. 1, 1-5 (1989).
104. Kawagishi T, Nishizawa Y, Emoto M et al. Impaired retinal artery blood flow in IDDM patients before clinical manifestations of diabetic retinopathy. *Diabetes Care* 18, no. 12, 1544-1549 (1995).
105. Grunwald JE, DuPont J, Riva CE. Retinal haemodynamics in patients with early diabetes mellitus. *British Journal of Ophthalmology*, 80(4), 327–331 (2006).
106. Grunwald JE, Riva CE, Baine J, Brucker AJ. Total retinal volumetric blood flow rate in diabetic patients with poor glycemic control. Investigative Ophthalmology & Visual Science, 33(2), 356–63 (1992).
107. Bursell S, Clermont AC, Kinsley BT, Simonson DC, Aiello LM, Wolpertf HA. Retinal Blood Flow Changes in Patients With Insulin- Dependent Diabetes Mellitus and No Diabetic Retinopathy. *Investigative ophthalmology & visual science*, 37(5), 886–897 (1996).
108. Riva CE, Grunwald JE, Sinclair SH, Petrig BL. Blood velocity and volumetric flow rate in human retinal vessels. *Investigative ophthalmology & visual science* 26, no. 8 1124-1132 (1985).
109. Murray CD. The physiological principle of minimum work: I. The vascular system and the cost of blood volume. *Proceedings of the National Academy of Sciences of the United States of America* 12, no. 3, 207 (1926).
110. Feke GT, Tagawa H, Deupree DM, Goger DG, Sebag J, Weiter JJ. Blood flow in the normal human retina. *Investigative ophthalmology & visual science* 30, no. 1, 58-65 (1989).
111. Garcia Jr Julian PS, Garcia PT, Rosen RB. Retinal blood flow in the normal human eye using the canon laser blood flowmeter. *Ophthalmic research* 34, no. 5, 295-299 (2002).
112. Guran T, Zeimer RC, Shahidi M, Mori MT. Quantitative analysis of retinal emodynamics using targeted dye delivery. *Invest. Ophthalmol. Vis. Sci*. 31, 2300–2306 (1990).
113. Hardarson SH, Stefánsson E. Retinal oxygen saturation is altered in diabetic

retinopathy. *British journal of ophthalmology* 96, no. 4, 560-563 (2012).
114. Khoobehi B, Firn K, Thompson H, Reinoso M, Beach J. Retinal Arterial and Venous Oxygen Saturation Is Altered in Diabetic Patients. *Investigative ophthalmology & visual science* 54, no. 10, 7103-7106 (2013).
115. Hammer M, Vilser W, Riemer T et al. Diabetic patients with retinopathy show increased retinal venous oxygen saturation. *Graefe's Archive for Clinical and Experimental Ophthalmology* 247, no. 8, 1025-1030 (2009).
116. Cai J, Boulton M. The pathogenesis of diabetic retinopathy: old concepts and new questions. *Eye* 16, no. 3, 242-260 (2002).
117. Jørgensen C.M, Hardarson, SH, Bek T. The oxygen saturation in retinal vessels from diabetic patients depends on the severity and type of vision-threatening retinopathy. Acta Ophthalmologica, 92(1), 34–9 (2014).
**** Very thorough and complete experiment, which takes into account many groups from normal subjects to advanced diabetic retinopathy patients with limitations as well**

ACKNOWLEDGEMENT

This is an Author's Accepted Manuscript of an article published in:
"Leontidis, Georgios, Al-Diri, Bashir, and Hunter, Andrew. "Diabetic retinopathy: current and future methods for early screening from a retinal hemodynamic and geometric approach." *Expert Review of Ophthalmology* 9.5 (2014): 431-442."

[Copyright Taylor & Francis], available online at:
http://www.tandfonline.com/eprint/AXMGZqvwH4u7PeBQixxa/full
and
http://www.tandfonline.com/doi/full/10.1586/17469899.2014.945521

[**DOI:** 10.1586/17469899.2014.945521]