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### **Review Article**



Diabetes Mellitus Associated Progressive Neurovascular Retinal Injury: Recommendations for Imaging and Functional Testing and Potential Role for Early Intervention with Modern Retinal Laser Therapy

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### ABSTRACT

Diabetes mellitus is now recognized as a systemic, autoimmune, microvascular disorder, which, in the retina results in severe, multifocal injury now recognized as a leading cause, world-wide, of progressive vision loss. To address this problem in the prediabetic and overt diabetic states, it must be realized that, although the injury processes may be system wide, there are varying responses, effector, and repair mechanisms that differ from organ to organ or within varying cell structures. Specifically, within the "neurovascular unit" of the retina, lesions occur of focal microvascular occlusions, inflammatory endothelial and pericyte injury, and small vessel leakage resulting in injury to astrocytes, Müller cells, microglia, and causing progressive neuronal apoptosis. Such lesions are now recognized to occur before the first microaneurysms are visible by fundus camera imaging or before detectable symptoms or signs recognizable to the patient or clinician. Treatments, therefore which currently are not initiated until edema develops or progressive vascular occlusion, are applied relatively late with some reduction in progressive cellular injury but with minimal vision improvement.

Desperately needed are newly developed imaging and functional testing methods that detect the early stages of microvascular injury and neuronal apoptosis when the processes are potentially reversible. Treatment, when applied at such early stages, therefore can preserve far better functional vision. However, to be acceptable, such interventions must be minimally invasive with patient appreciated improvement in vision. Micropulse applications of laser retinal phototherapy appear to offer treatment for such early intervention, and the mechanisms are discussed herein, not just to reverse the early alterations, but also for later stages of the progressive neurovascular and vision degradation when patients will continue to present.

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### Introduction

There are 463 million persons worldwide with diabetes mellitus, representing 9.3% of the adult population. This is expected to double in the next 25 years [1], with approximately 37% of US adults older than 20 years and 51% older than 65 having prediabetes with predicted high rates of conversion to type 2DM with aging [2]. Because of this, prediabetes, along with diabetes, are now recognized as a major risk factor for the development and progression of retinal, optic nerve, and brain neurovascular dysfunction [3-8], resulting in severe vision loss [9,10]. The focus of the medical industry predominantly has been to examine the effect of hyperglycemia control along with other aggravating comorbid factors (hypertension, hyperlipidemia, smoking, obesity, and sleep apnea) on the progression of large vessel occlusive disease, because of the rising incidence of stroke and myocardial

infarction [11,12,13]. However, it is now recognized that the small vessel disease is 20-30 times more common, resulting in severe disabling neuronal injury with resultant progressive vision and cognitive impairment [14-21].

To address this problem, it must be realized that, although some of the injury processes associated with diabetes are system wide and, therefore, possibly amenable to systemic therapy, there are varying response, effector, and repair mechanisms that differ from organ to organ or within varying cell structures within an organ tissue. For instance, inflammatory processes in the pancreas that kill the beta cells, resulting in reduced insulin production, are different from those in the adipose tissue that increase insulin resistance and are also different from responses within tissues of neurons, astrocytes, microglia, pericytes, and endothelia of the retina and brain, now termed the "neurovascular unit" and certainly with genetically defined variable susceptibility [22].

## The Normal Human Retinal Neurovascular Unit and the Imaged Lesions Associated with Diabetes

The inner retina, as well as the subcortical, deep, white matter of the brain share a common organization of the microvasculature supplied by small, perforating, independent vascular units of similar components, that demonstrate little ability to provide collateral flow [23,24]. Furthermore, they share an intensive balance between tissue metabolism and vascular regulation of blood flow, termed autoregulation. In the retina the local precapillary arterioles are very sensitive to local extravascular oxygen levels. Because of the tendency of the intense light focused on the retina to potentially generate oxygen-free radicals within the tissue [25], the blood flow is maintained at critically low oxygen levels, therefore requiring extremely careful regulation of flow, moment to moment, despite changes in arterial inflow or venous outflow pressures. While the choriocapillaris supply to the outer retina manifests a high flow rate to provide high oxygen delivery for the intense oxygen consumption of the immediately overlying photoreceptors as well as a stabilizing heat sync, little oxygen diffuses through into the middle and inner retina. At night the photoreceptor and middle nuclear layer neurons are hyper-polarized (termed "dark current") in order to reduce spontaneous transmissions (flashes of light), thereby demanding sensitive adjustment of flow. In humans, noninvasive flow measurements allow assessment of baseline flow by laser doppler velocimetry along with autoregulatory changes induced by increased or decreased inhaled oxygen and perfusion pressure [26].

Astrocytes in the inner retina synapse on blood vessels to maintain such autoregulation [27], while Müller cells, which span all retinal layers, coordinate the vascular responses to meet the metabolic demand of neurons, interchange metabolites, recycle neurotransmitters and glutamate, and control extracellular ion homeostasis. Microglia, which normally reside within the plexiform retinal strata, exhibit ramified processes responsible for immune surveillance along with monitoring noxious insults such as oxidative stress, hypoxia, or inherited mutations, that trigger proliferation and migration to the sites of injury [28,29]. Coordinated activity among neurons, Müller cells, astrocytes, and microglia, together with the microvasculature, is essential for the maintenance of normal metabolic function and visual perception. Although the local autoregulatory mechanisms preeminently attempt to maintain flow aligned with metabolism, it is recognized that certain conditions, such as peripheral limb, coldinduced vasospasm also produce coexistent transient reductions in retinal blood flow from focal arteriolar vasospasm in susceptible individuals (termed systemic vascular dysregulation, SVD) [30]. Although these are thought to be transient, SVD has been associated with a risk for anterior ischemic optic neuropathy, retinal venous occlusion, central serous chorio-retinopathy, and especially for glaucomatous disc nerve-fiber-layer loss [31,32]. More recently SVD has been associated with a more chronic, reduced parapapillary, microvascular density in what were thought to be normal eyes [32]. This may be only one manifestation of the system-wide disorder that alone, or perhaps in conjunction with other abnormalities, result in more prolonged injury to the microvasculature.

The coordination of blood flow to metabolic demand, synaptic activity, and waste removal is coordinated through neurotransmittermediated signaling, particularly through glutamate release of nitric oxide from neurons and of arachidonic acid derivatives from astrocytes (and possibly from neurons) and by ATP conversion to adenosine [33-35]. The blood-retinal barrier is formed by the continuous microvascular endothelium and its underlying basement membrane with pericytes that tightly encircle the endothelium, and astrocytes in the surrounding tissue space that extend their cell processes towards the endothelium and insert on the basement membrane. Pericytes are noted to be highly susceptible to damage in ischemic conditions (when ATP levels are low) suggesting the possibility that pericytes are the cause of the constricted capillaries at the start of a stroke [36] as they remain in rigor (because no ATP is available to relax their contractile filaments), causing the capillaries to remain too narrow for the passage of blood cells, predominantly leukocytes. In agreement with this, pericytes are noted to remain constricted even for hours after the re-opening of an occluded parent artery (in brain models [37]) that results in endothelial damage and capillary leakage. Suppression of oxidative and nitrosative stress prevents this pericyte constriction, restoring the patency of capillaries and tissue recovery [37]. This has important implications to the understanding of tissue reactions to prolonged ischemia due as well to small vessel disease, as well as with the occurrence of vacillations in oxygen levels or in blood pressure (as occurs with sleep apnea in which a sudden drop in oxygen induces autoregulatory dilation of the retinal arterioles resulting in capillary hypertension that is severely aggravated with the sudden rise in blood pressure that occurs at the "reprise" end of each apneic episode and has been associated with capillary occlusion even in normal, non-diabetic individuals suffering sleep apnea [38]).

The mechanisms by which the retinal microvasculature in the diabetic individual is more susceptible to large, as well as small vessel abnormalities of flow regulation are now better understood. In the human diabetic eye increased blood flow has been measured associated with elevations of serum glucose, occurring as well in the eye of the "prediabetic" with variable hyperglycemia [39]. This results in capillary hypertension [40,41] along with a reduced capability of autoregulation, [26,41-43], apparently worse in the middle retinal layer than that in the inner, more superficial layers [44], but, in both, due to abnormalities of the nitric oxide (NO) messenger within the arteriolar musculature [45]. These mechanisms, as well the recognized additional factors of hypertension, smoking, sleep apnea, and others that will be discussed below, result in aggravation of both retinal small vessel ischemic lesions in the diabetic and prediabetic populations [46,47] with progressive vision loss (and in the brain which shares similar outcomes resulting in microvascular cognitive impairment and dementia [48]). While arteriolar oxygen reactivity and its match with metabolism have been the primary focus of investigations into the aberrant cause of the retinopathy, it is also now recognized that toxic byproducts accumulate in the interstitial, perivascular space resulting in neuronal apoptosis. Such toxic products have been thought to be eliminated primarily via venular outflow. However, more recently perivascular fluid outflow has been demonstrated to exit also via the lamina cribrosa and a hypothesized glymphatic CSF drainage [49]. While the importance of this aspect of toxic removal is now better recognized in the pathogenesis of glaucomatous nerve fiber layer injury, its causation in other retinal and brain neurodegenerations at this time remains only hypothetical.

Diabetic retinopathy (DR) has traditionally been considered to be a microcirculatory disease caused by the deleterious metabolic effects of hyperglycemia per se and the metabolic pathways triggered by hyperglycemia, including the polyol [50], hexosamine, and DAG-PKC pathways [51,52], which result in advanced, glycation end-products [53] and the induction of oxidative stress [54]. The primary retinal lesions, however, are now recognized as small neurovascular lesions composed of

both focal vascular occlusions mixed with varying degrees and types of inflammatory endothelial and pericyte injury. These produce small vessel leakages that result in injury to structural astrocytes, Müller cells, and microglia, with both processes causing progressive focal neuronal apoptosis that has been noted to occur in both prediabetic and diabetic individuals. The earliest structural change appears to be a loss of microvascular pericytes via apoptosis or migration, which then leads to weakening of the blood-retinal barrier [55] through loss of the inter-endothelial tight junction proteins [56]. This precedes, but results as well, in apoptosis of the endothelial cells, resulting in loss of endothelial nitric oxide (NO) production. NO is the primary vasodilator that provides for the normal small arteriolar autoregulatory capabilities discussed above. Furthermore, capillary hypertension, caused by the elevated flow levels in the remaining patent vessels, is recognized to stimulate the endothelial cell production of intercellular adhesion molecules (ICAMS) that slow and obstruct the normal passage of WBC's through those capillaries [57] with subsequent breakdown and endothelial injury [58,59]. Studies of retinal vessel oxygen saturations indicate a reduction in oxygen delivery, confirming such focal occlusions with increased flows that occur within the surrounding microvasculature units early within diabetics [60].

However, for the past 50 years the clinical focus for detecting and grading diabetic retinopathy (DR), as well as considering the instigation of treatment, has been the viewing on examination of secondary retinal vascular lesions of microaneurysms, hemorrhages, intra-retinal and epiretinal microvascular proliferation, arterial wall thickening and venular irregular dilation, with varying degrees of intracellular and extracellular edema and irregularly scattered lipid [61,62]. Of greater significance, is that neuronal and Müller cell death occurs in focal patterns within multiple retinal layers much earlier (in both diabetic and prediabetic individuals) [63,64], even prior to the pericyte and endothelial cell apoptosis [64,65-69]. These alterations, however, are not visible on examination or appreciated with standard imaging by fundus cameras or ocular coherence tomography (OCT) until there is fairly severe, diffuse neuronal death and atrophy that is appreciated structurally as progressive thinning on OCT of the inner retinal nuclear layers and nerve fiber layer [64,66-68,70-72]. This occurs in 20% of diabetics even prior to observation of microaneurysms [71] and in prediabetics as well [73]. In addition, the Müller cells (macroglia) and microglia appear to play key roles in what is considered to be an inflammatory process [74] via activation of their endoplasmic reticulum from the stress of hyperglycemia [75]. The Müller cells express vimentin and glial fibrillary acidic protein (GFAP) that inhibit both neural [76,77] and capillary regeneration [78]. Müller cells are now also recognized as living optical fibers that guide red and green light through the inner retinal tissue to specialized cone photoreceptors [79], minimizing intra-retinal light scatter to support high resolution vision [80,81]. Therefore, reactive gliosis of and by Müller cells [74] contributes to the early visual abnormalities that have been detected in diabetic subjects via multifocal ERG [82-84], as well as when tested at fixation with blue-sensitive acuity or contrast sensitivity at low light levels [65, 85,86] or with resolution perimetry conducted under low illumination, low contrast conditions [87].

Unfortunately, these functional and electrophysiologic tests are seldom performed in the standard ophthalmologist's or optometrist's office and are not recommended in current evaluation guidelines [88], resulting in the relatively late discovery of injury when macular edema or the more severe lesions of DR are recognized [89]. This delays intervention, currently consisting primarily of repeated intravitreal injections of antiVEGF or steroid medications that result in marginal vision improvement. In multiple studies, only 25–34% demonstrate improvement of  $\geq$ 3 ETDRS lines of high contrast, photopic visual acuity, and primarily only among the eyes with severe retinopathy and poor initial vision, and with 23% considered non-responders and 27% having only a moderate response [62,90-93]. Recent methods of OCT derived microvascular analysis (termed OCTangiography, OCTangio) have provided analysis of the retinal microvasculature. These demonstrate that reductions in microvascular density occur prior to the development of the traditional retinopathy lesions and progressively worsen over time, correlated with worsening grades of the retinopathy [94-97]. However, the microvascular disease is not uniform through the retinal layers, but occurs in a focal process primarily or earliest within the superficial ganglion cell layer with a slower decline in the deeper internuclear, plexiform, and middle neuronal layers [95-98], as well as within the radial peripapillary capillaries of the nerve fiber layer [99]. However, owing to the limitations of this new imaging modality and the great variety of algorithms utilized for vascular layer segmentation and flow evaluation, much debate continues over which vascular plexus is primarily affected, especially within the parafoveal area of the central vision where the layers merge [100]. No consensus has been reached on the ideal set of quantitative metrics, whether parafoveal vascular density or total avascular area or a derivative "adjusted flow index", that would best quantitate microvascular occlusive progression [98] as well as the adequacy of the remodeling that is known to occur [101]. As well, it must be understood that flows and diameters are not quantitated, only the microvascular grid structure. In addition, the indices derived have represented averages within the perifovea; it must be recognized that diabetes is a focal microvascular disease process, and evaluations must reflect this.

Simultaneous with the microvascular alterations, there is neuronal apoptotic injury with death and tissue fluid that appear as darkened regions on adaptive optics scanning laser ophthalmoscopy [101] and then with eventual diffuse atrophy of the various layers noted on OCT beta scans as discussed above [95,97,102-105]. It remains unknown whether the microvascular occlusions, with degradation of the endothelia, pericytes, and Müller cells occurs prior to, and causes the neuronal apoptosis, or whether the microvascular changes are the consequence of prior neural tissue inflammation and proximal cellular injury. It is now recognized that the current functional testing and imaging methods fail in this regard. What is required are improved methods to detect and grade the progression of both processes that compromise the neurovascular integrated function with then the hopeful development of treatments applied early in the course, before the irreversible death and atrophy [87,96,106,107].

### Pathologic Mechanisms Implicated in the Development of Retinal Lesions In Diabetic Patients

Diabetes mellitus, therefore, is now recognized to involve a systemic, autoimmune, inflammatory disorder causing focal microvascular occlusions and alterations of the blood-retinal and blood-brain barriers, that occur in the "pre-diabetic" as well as diabetic individuals [1,87,108,109]. In the Diabetes Prevention Program (DPP), 7.9% of subjects with impaired glucose tolerance had retinopathy [110], similar to the 8.1% prevalence of retinopathy observed among individuals with prediabetes in the Gutenberg Health Study [111] with the variability of the glycemia a significant recognized risk factor for both development

and progression [112,113]. HbA1c has been the primary criteria differentiating the diagnosis of diabetes from prediabetes, but it must be stressed that there are many well-characterized "pitfalls" of this measurement [114]. Although DR or its progression is recognized to be related to HgbA1c or associations with HTN and hyperlipidemia, studies across the globe have observed that the risk of many of the associated co-morbidities are the same in diabetics and prediabetics and affect all age groups indicating these are complicated interactions [5]. Across the striae of hyperglycemia definitions, the increasing requirement for insulin, brought about by the progressive insulin resistance of peripheral fat, together with the pancreas Beta cell loss (both a product of immunologic reactions) is associated in itself, with aggravated risk of vascular induced retinal as well as cognitive dysfunction [115].

### Microvascular Injury, Dysregulation, and Occlusion

One proposed mechanism of vascular injury associated with diabetes is through impairment in the regulation of the transient receptor potential cation channel (TRPC). Under physiologically healthy conditions, the vascular wall responds to high pressure by upregulating TRPC, triggering reactive vasoconstriction. However, continuous stimulation ultimately results in poor flow autoregulation to the variations of the stimulant (studied in the brain) [116]. Both aging and deficiencies in insulin-like growth factor 1 (IGF-1) in the presence of hypertension have been shown to impair upregulation of TRPC [117,118], a proposed mechanism of vascular injury associated with diabetes[119]. Failure to upregulate TRPC leaves the microcirculation vulnerable to damage caused by the variation of pressures within the microvasculature resulting in increased blood-retinal and blood-brain barrier leakage and neuroinflammation [120,121]. There is also a gradual accumulation of multiple molecular fractures of the intima and internal, elastic lamina of the microvasculature. The accumulation of such patches eventually transforms the elastic lamina to a stiffened, friable wall. The resulting array of structural, degenerative events in the vessel wall, including death of endothelial cells, basal membrane thickening, and atrophy of the smooth muscle cells [122,123] all lead to rupture (microbleeds), microinfarcts, and loss of tight junctions with reduced retinal barrier integrity. In addition, within the retinal capillaries of the diabetic, leukocyte drag and adhesion occur due to leukocyte elevated stiffness and the upregulation of endothelial derived adhesion molecules (ICAMS and VCAMS) that adhere with the leukocyte integrins resulting in the adherence of the leukocyte to the endothelium and degradation producing focal microvascular occlusion and leakage [124-126]. The resultant increased permeability of the blood-retinal barrier then results in interstitial accumulation of water, gliosis, and cytokine proteins with further worsening of inflammatory/oxidative stress events that damage the parenchyma. Pericytes and oligodendrocytes are extremely vulnerable to these ischemic and toxic insults [127-128] also resulting in impaired normal tissue repair [129].

# Inflammatory Mechanisms Associated with the Neurovascular Injury

Systemic inflammatory mechanisms certainly appear active in a number of the recognized neurodegenerative processes of diabetes, with microglia and astrocytes as the primary contributors to the inflammation. Normally quiescent, such activated cellular components begin to secrete cytotoxic substances that contribute to neuronal cell death [103]. As discussed above, in the diabetic or prediabetic patient, there is chronic, systemic, low-grade inflammation [87] that is reflected by high levels of serum cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ), C-reactive protein (CRP), and the interlukin cytokines IL-6, IL-8, IL-1B, and the receptor antagonist, C5a [130-131] along with granulocyte and

monocyte elevations [130,132]. While this inflammatory state is thought to be the mechanism by which metabolic disorders such as diabetes are associated with the development of small vessel organ failure, there is significant variability of the cytokines that indicates a complex process among the various organ tissues [133-137].

As discussed above the neuronal apoptosis within the inner retina appears preceded by earlier loss of the microvascular pericytes and then endothelia, resulting in loss of reactivity with very early vasodilation along with early increased permeability [40,55]. Chronic, variable hyperglycemia has been observed to cause an increase in release of glutamate with loss of neuroprotective factors due to oxidative stress with the accumulation of the waste products of glycolysis that trigger the apoptosis [65]. However, we must acknowledge that all of the supporting structures of the neurons, including the Müller cells, astrocytes, pericytes, and infiltrating monocytes (microglia), have also been defined as critical participants in the neuroinflammatory and protection processes[138]. Activated Müller cells mediate both protective and detrimental effects upon the ganglion cell layer neurons of the retina through a variety of receptors that result in the expression of multiple factors that affect neuronal survival [139] including glial fibrillary acidic protein (GFAP), vimentin [140,141] and growth factors, such as brain-derived neurotrophic factor (BDNF) and platelet-derived growth factor B (PDGF-B) [64,74,142]. Müller cells also manifest a protective role by absorbing glutamate, that reduces the cytotoxic effects of extracellular glutamate levels [143,144]. Diabetes not only reduces the Müller uptake of glutamate within the neuro-retina [145] but also reduces the activity of the enzyme, glutamine synthetase, hindering the ability to convert the excess glutamate to inactive glutamine [146] or to oxidize the glutamate to a-ketoglutarate [143]. Activated Müller cells, therefore, serve not only as rapid sensors of neuronal damage to initiate repair and neural regeneration [147,148], but they may also initiate a deleterious inflammatory process with release of proinflammatory cytokines, such as activating transcription factor 4 (results in release of ICAM-1 and VEGF [75,149]) as well as TNF $\alpha$ , IL-1, and other cytokines known to exacerbate apoptosis of adjacent neurons [69,139,150]. Such cytokines, as mentioned above, have been detected in aqueous humor samples of diabetic eyes with varying grades of retinopathy [151,152]. This dual action, therefore, is certainly a double-edged sword and should be accounted for in the design of therapeutic approaches addressed to abrogate Müller glial activation.

Microglia within the retina are the principal immune effector cells, constantly surveying their environment in preparation to react to insult or injury. When activated, for example in diabetes, microglia become ameboid, and migrate within the inner and outer retina, releasing pro-inflammatory and vasoactive substances, contributing to the local inflammatory response that results in increased vascular permeability. The microglia are normally phagocytic, clearing damaged myelin to allow neuronal repair. However, a chronic pro-inflammatory state, such as that induced with variable hyperglycemia, alters the response, preventing remyelination. In human diabetic retinas, activated microglia are often observed associated with the vasculature as discrete hyper-reflective foci on SD-OCT [153], leading to the term "microglial perivasculitis", even in diabetic eyes without clinical signs of DR [154] and which result in the release of numerous cytokines, including, VEGF, IL-6, IL-1B, TNFa, and monocyte chemoattractant protein-1 (MCP-1) with several studies indicating the contribution of these to the microvascular permeability and occlusive complications as well as progressive neuronal apoptosis

[64,155]. It should be noted, however, that activated microglia may also demonstrate a neuro-supportive function, similar to Müller cells, producing anti-inflammatory and neurotrophic factors, (e.g. IGF-1, BDNF, and GDNF, among others [152,156-158]). The detection of microglial activation ultimately may prove of value in early disease diagnosis, as modulation of microglial responses appears to provide the ability to alter disease progression.

### **Treatment of Diabetic Retinopathy**

As discussed above, while the Diabetes Control and Complications Trial and the United Kingdom Prospective Diabetes Study demonstrated that controlling blood glucose, as measured by HgbA1c and periodic blood glucose levels, reduced the progression of developed complications such as DR, [159,160], post-hoc analysis of the DCCT data has revealed that only 11% of the risk in retinopathy development could be attributed to hemoglobin A1c. Later studies showing significant, perhaps even greater impact of the glucose fluctuations [161], as well as the degree of insulin usage in type 2 DM with insulin resistance [162]. As discussed above, the systemic microvascular disease that causes the progressive, profound, microvascular occlusion and inflammatory injury is now recognized to exist in the prediabetic as well as the overt diabetic, especially among those demonstrating insulin resistance with hyper and hypoglycemic variability [163]. This accounts for a number of the drugs that were initially approved for the control of hyperglycemia, now recognized to have significant, but variable effects on the systemic as well as individual end-organs. However, the clinical treatments, whether systemic or specifically directed to the retina, have been limited by the traditional evaluation methods of imaging and/or functional organ testing and only following stages of significant and often severe organ failure. The above cited microvascular and neuroparenchymal cellular pathologies represent a complex array of processes that are targets for earlier diagnosis and treatment. This review will focus primarily on the effects of traditional and newer methods of retinal laser treatment upon the progressive neurovascular injury caused by diabetes as well as potential methods for initiation earlier in the course when microvascular injury and neuronal apoptosis are reversible. For a more comprehensive discussion of traditional hyperglycemia treatments as well as newer systemic treatments currently available or under clinical evaluation for treating the retina as well as brain neurovascular occlusive and inflammatory injury the reader is referred to these excellent review articles [87,164-168].

New methods of imaging of the focal neuronal apoptosis and inflammation within the retina (via fluorescent staining of Bis(zinc-dipicolylamine), Zn-DPA) are in progress [169,170] and appear to enable earlier detection of neuronal injury during the apoptosis process when reversible. This will then empower initiation of treatments before the death and severe, diffuse atrophy that is the current threshold. Improvements in the OCTangio device scanning times have improved the evaluation of flow characteristics, and with improved sectoral microvascular lumen reconstruction will enable identification of the early focal abnormalities (perhaps along with analysis of retinal capillary white blood cell flow densities utilizing the non-invasive blue field, entoptic phenomenon [57,125]). Whether these preclude or are associated with the focal neuronal apoptosis is for evaluation in the future as new methods now allow the functional testing to be overlaid onto the neuronal and microvascular occlusion lesion imaging. It is imperative, therefore, that diabetics and prediabetics be screened regularly, utilizing improved imaging as well as functional testing of resolved targets at fixation [106,164] and the central visual field utilizing resolution targets under conditions of reduced contrast and luminance with fixation control [87] to define earlier, reversible disturbances.

Once discovered and tracked, however, the difficulty remains as to how to address the progressive neurovascular injury processes. It is axiomatic in medicine that the earlier an effective intervention can be applied, the better the outcomes [165]. Certainly it is easier to prevent the severe complications of disease, such as severe vision loss from DR, than to attempt to restore normal form and function once it has been lost. Ethically, early, preventive treatment must be, not only effective, but extremely safe and maximally free of adverse treatment effects. This is because treatment may need to be continued for many years, during which time the impact of adverse effects are increasingly likely to occur. Practically, this is also because patients with preclinical disease are generally asymptomatic and, from a self-perceived, point of view, functionally normal, although often modified by adaptation,. Putting either at risk is unacceptable to patients who tend to place more value on their current state of being than upon a hypothetically better future that might only be attained through inconvenience and pain [171,172]. As will be realized in the following, such real considerations disqualify most therapeutic treatment options from consideration as preventive treatments for DR.

The Traditional Approach to Treatment of Diabetic **Retinopathy: Continuous Wave Laser Retinal Photocoagulation** The apocryphal origins of retinal photocoagulation for the complications of diabetic retinopathy were the observations that eyes with large chorioretinal scars occurring in one eye, such as from congenital infections, appeared to be somewhat protected from DR compared with their fellow eyes. Retinal photocoagulation, accomplished by xenon arc or laser, was recognized as an effective method of producing chorioretinal scarring, and on this basis, was applied to prevent the vision destructive complications. [168,173], The mechanism for the laser effect was not known but presumed to be associated with reduction of retinal ischemia, hypothesized to possibly also result in increased oxygen tension by thinning the retina and increasing diffusion of oxygen from the underlying choroid. Based on this observation, 500um, full thickness burns were placed in the periphery (Figure 1), as far anterior as possible, preferably to the ora serrata, and within all areas posteriorly of absent capillary flow visualized on fluorescein angiography, sparing the posterior retina so as to preserve central visual function, and with the burns placed approximately 0.5 DD apart because of the postoperative spread of the deep retinal and RPE scars, hopefully retaining some peripheral vision.



**Figure 1:** Color fundus photograph of left fundus 2 weeks after the application of peripheral, pan-retinal laser photocoagulation with 500 um applications demonstrating nearly contiguous lesions applied peripheral to the disc and posterior vascular arcades [from: https://www.cairnseye.com/].

Pan-retinal scatter laser photocoagulation was demonstrated to reduce the incidence of severe vision loss due to complications of the epiretinal neovascularization from 16.3% to 6.4%, but was associated with some further decline in central acuity often by 2 or more ETDRS lines in nearly 50% [174]. Because the pan retinal laser was found to be superior to no treatment, it became widely adopted and employed for advanced stages of proliferative retinopathy to prevent vitreous hemorrhage and tractional complications including traction retinal detachment. Secondary complications of the initiation or aggravation of macular edema that occurred due to inflammation resulting from thermal tissue destruction [175,176] or from macular distortion that occurred because of the fibrosis and contracture of the neovascularization during involution that is now recognized to also be a result of the inflammation. Furthermore, by the time the neovascularization was noted on examination or resulted in visual symptoms from a vitreous hemorrhage or traction distortion of the macular retina, the capillary non-perfusion had proceeded sufficiently posterior such that, with the burns destroying all layers of retina and underlying choroidal vasculature, the peripheral visual field was severely constricted, often to within 10-15 degrees from fixation, and with little alteration of the ongoing progressive macular OCT inner retinal microvascular loss and neuronal atrophy [177].

When macular edema was recognized as a common cause of vision loss, the application of focal laser photocoagulation, utilizing smaller, focal applications of full thickness burns was evaluated and, with the publication of the multiple reports from the Early Treatment Diabetic Study in 1987 [159], became the standard of care. Although the retinal edema, as measured by central retinal thickness or retinal volume, with or without cysts, was improved, improvement in central chart acuity was minimal, Furthermore, the parafoveal laser burns were associated with severe destruction of retinal photoreceptors and with progressive enlargement of the laser retinal scars inward and laterally [178], lead frequently to foveal involvement or to secondary choroidal neovascularization and subfoveal fibrosis [179], However, the macular scarring was largely ignored, because of the use of chart acuity as the primary functional outcome. As discussed above, with macular disease, when charts are used to conduct any testing of central vision, the patient moves their eye searching for the

best functioning area regardless of scotomas. When parafoveal full thickness burns are produced and result in significant paraxial visual field defects [179,180]], they result in non-trivial, adverse consequences, including, poor reading speed and ability, loss of night vision, loss of driving privileges, and disruption of circadian rhythms and normal sleep cycles, to name but a few [181-183]. As destructive-sparing alternatives to photocoagulation became available (e.g. intravitreal injected anti-VEGF compounds and steroids), these drawbacks relegated laser treatment to lower tiers (initiated after intravitreal injections) or avoided options [19,184]. The frequency of the required intraocular injections, however, has restricted patient acceptance of these treatments, limiting the results and has forced the industry to turn to prolonged delivery ports, which require surgical implantation and have been associated with significant complications, including cataract, extrusion, and endophthalmitis [185], reducing the benefit/risk ratio. Such factors, therefore, preclude the consideration of either retinal photocoagulation or intravitreally injected drug therapy as early and / or preventive treatment of DR in its earliest stages of development when the injury is reversible and most amenable to treatment.

### **Current Retinal Laser Therapy**

While Modern Retinal Laser Therapy (MRT) is laser treatment of the retina similar to conventional photocoagulation, it approaches disease and treatment in a fundamentally different manner. MRT is the result of the discovery in 2000 that laser-induced retinal damage (LIRD), the single cause of all SAEs associated with conventional photocoagulation, was unnecessary for effective treatment. [181,186-189]. Rather, thermal laser photostimulation of the RPE, at levels sublethal to the retinal pigmented epithelium (RPE) and neurosensory retina (NSR), resulted in improved and normalized retinal function without adverse treatment effects [190,191].

Furthermore, the effects of photothermal stimulation of the retina appeared to be agnostic to the cause of the underlying retinal dysfunction, thus allowing MRT to normalize retinal function for a number of identified chronic progressive retinopathies (CPRs). [192,193]. The action of MRT appeared to be a "non-specific trigger of disease-specific repair" through a physiologic, healing "reset" of the dysfunctional retina to normalize retinal function [191,194].

If one were to attempt to prevent diabetic retinopathy using a targeted approach, the preceding review of the pathologic changes, which occur in the retina prior to the development of traditional clinical signs of retinopathy, makes clear that there are a great many potential treatment targets currently known, and almost certainly additional that remain unknown. Thus, targeted drug or biologic therapy to prevent DR in the face of ongoing DM multi-dysfunction must confront the daunting challenges for any targeted therapy, rendering development of such a therapy - one that is both effective and without major adverse treatment effects - highly unlikely [195]. It should be noted as well that most of the potential targets reflect aspects of cellular dysfunction that then lead to further dysfunction, often in a vicious cycle. Rather than targeting the secondary effects of cell dysfunction as is attempted with current retinal biologics, MRT addresses the cause of these effects, the DM-induced cellular dysfunction itself [194,196].

All CPRs, including DR, result in progressive neuronal dysfunction and cell death, and while each may have different proximal causes, the particular stress placed on susceptible cells from these different diseases result in a common effect of

excessive protein misfolding, aggregation, and resultant cellular dysfunction. A potential responsive healing process, known as the unfolded protein response (UPR) of the endoplasmic reticulum (ER) within the cell is responsible for proteostasis and maintainance of normal cell function. [197-200]. Cell growth, gene mutations, environmental stressors (including DM), infection, reactive oxygen species (ROS), and aging all tax these cellular proteostasis mechanisms wherein the peroxisome proliferator activated receptor  $\gamma$  (PPAR $\gamma$ ) plays a master gate keeper roll both in the injury and repair[201]. If this dysfunction exceeds the capacity of the UPR to un-fold and disaggregate misfolded proteins, as well as the ERAD (endoplasmic reticulum-associated protein degradation process) the UPR will then initiate cellular apoptosis, with ultimately, tissue failure [202].

Acting as "chaperones" that identify, transport, modulate, and aid the UPR function, "heat shock proteins" (HSP) particularly HSP 70, that are upregulated by photothermal stimulation and which bind immunoglobulin protein (BiP), are critical for cell homeostasis and proteostasis [203]. In neurodegenerative diseases, the gradual failure of the homeostatic capability of the UPR occurs, eventually resulting in cell death [199]. Such gradual failure that is characteristic of CPRs appears to result because of poor stimulation of the protective HSP activation and the UPR with inadequate generation of HSP BiP.[204]. Thus, in such chronic diseases the reparative system itself progressively fails [205-208]. Acute stressing of the UPR, modulated by HSP activation, however, improves UPR function with upregulation of protein repair and improved cell function and survival. [200]. Sudden temperature elevations, as produced by MRT appear the most effective and efficient activators of the HSP-initiated restoration of normal proteostasis, resulting in many effects that include improved mitochondrial function, energy production and utilization[197,209-210]. MRT exploits the thermal reactivity of the RPE to upregulate and generate HSP's, and thence improve the ER UPR to normalize cell function and thus inhibit progressive apoptosis [194,196,199]. This appears accomplished by inducing a temperature sensitive conformational change in HSP 70 via the k10 step in the thermal activation kinetics that accelerate protein refolding and processing 35% over baseline levels in dysfunctional cells, defined as those with high levels of misfolded proteins, but not in normal cells.[211] Thus, if both dysfunctional and normal RPE cells are directly exposed to MRT, the only effect will be normalization of the abnormal cell. This describes the "patho-selective" function of MRT [194]. Within in vitro, in vivo, and in human studies, MRT RPE HSP activation results in a wide range of restorative effects that result in improved cell function, normalization of RPE cytokine expression and response normalization of retinal Mueller cell function, producing increased markers of reparative acute inflammation and decreased biomarkers of chronic inflammation, with reduced ROS, increased retinal NO, and local and systemic therapeutic immunomodulation. [152,153,193,212-217]. Thus, by sublethal, photothermal activation of the salvific HSP response, MRT restores normalized cellular function in a manifold of ways, including via improvement in the ER UPR, without regard to the underlying clinical cause of the protein misfolding and dysfunction. Finally, unlike photocoagulation and anti-VEGF drug therapy, MRT, employed with laser in the near infra-red 810nm wavelength, is inherently anti-inflammatory, allowing it to address the early neuronal inflammation in pre-clinical DR discussed above [218,219]. Rather than attempting to individually mollify the panoply of pathologic dysfunctions developing in the retina early in DM, MRT simply improves cell function through inherent repair. In doing this, MRT addresses the various dysfunctions homeotrophicly (returning toward normal). It does this safely with repeated efficacy when repeatedly applied, fundamental requirements of any potentially useful early or preventive treatment for a CPR such as DR [182,220]. In the following we will examine how MRT is used in the management of DR, and its suitability for early and preventive treatment as expressed in the requirements above.

### A Brief Review of The Biophysics of Sublethal Photothermal Retinal Stimulation

Rather than continuous wave (CW) laser application employed by photocoagulation and other inherently retina-damaging laser modes, such as 2R (Ellex, Inc, Adelaide, Australia) and SRT (Leutronix, Seoul, Rep. of Korea), the appropriate method of MRT employs a microsecond pulsed, solid-state laser (MPL) designed to preclude the possibility LIRD. While any retinal thermal stimulation is potentially therapeutic, reliable safety (avoidance of RPE damage) is the special province of MPL [181,190,191,221,222]. MPL applies pulses of laser energy between 40 and100us in duration, separated by "off" intervals of no transmitted energy. The frequency of pulsing, or the "duty-cycle" (DC) determines the duration of the "off" intervals [221]. If the off-intervals are of sufficient duration, inter-pulse heat accumulation is prevented, allowing fine control of laserinduced tissue hyperthermia time and degree. Studies have shown that DCs of 5% or less minimize the risk of inadvertent LIRD [191,211]. Use of longer laser wavelengths, such 810nm near infra-red, improve safety further because of reduced absorption within the heterogeneously and irregularly pigmented RPE of humans[211,220]. Using the correct combination of laser parameters, an exceptionally wide therapeutic range can be crafted allowing MPL to be performed using identical laser parameters on all eyes (without adjustment for eye-specific factors ) safely (well below the Arrhenius integral for thermal cell death) and effectively (well above the Arrhenius integral for thermal RPE HSP 70 activation) [191,211,220]. The clinical effect of this sublethal treatment to the retina is then maximized by en masse recruitment of abnormal RPE through the confluent treatment of large areas of the RPE. Panmacular low-intensity, high-density, subthreshold diode micro(second) pulsed laser (SDMTM) is the archetype that established and epitomizes the principles of MRT [187,190,220].

### Clinical Application of Modern Retinal Laser Therapy in Diabetic Retinopathy

It is axiomatic that for any treatment to be effective therapy, it should also be preventive, if provided sufficiently early. The paucity of preventive treatments in medicine is due the adverse effects that render most therapies unsuitable for that purpose. MRT, on the other hand, is both highly effective for treatment of DR and safe, without known adverse treatment effects. Further, the absence of LIRD means that MRT, unlike standard photocoagulation, is painless, increasing patient acceptance, a critical requirement for any early and /or preventive treatment. Thus, MRT appears well suited early treatment to prevent progressive DR and vision loss. As previously noted, because of the many adverse effects of conventional photocoagulation, intravitreal drug therapy has become the first-choice treatment for management of DME [91,184,223-225]. Unfortunately, virtually all large randomized controlled trials (RCTs), evaluating such injectables for relatively

severe DME, compared the drug to conventional laser retinal photocoagulation. In these studies, photocoagulation was generally more effective at eliminating DME, but the visual results of drugs were better (when restricted to the eyes with the severest initial loss). This illustrates the adverse effects of conventional laser photocoagulation LIRD applied in more advanced cases of DME. This is because such eyes are particularly prone to worsening results from the inflammation caused by the tissue destruction associated with photocoagulation. The precarious nature of advanced disease is yet another factor recommending early and/or preventive interventions when the retina is better auto regulated and functionally stable. Currently, avoidance of LIRD and increasing application of MRT principles for laser treatment of DME has become the norm when applying earlier thresholds for treatment application [220]. Several reviews and meta-analyses of MPL for DME have confirmed the superior safety and visual results vs conventional photocoagulation [181,187,226-233]. Applying MRT principles to MPL to preclude adverse treatment effects and maximize treatment efficacy, RCTs have shown laser to be comparable if not clinically superior to intravitreal anti-VEGF therapy, with a lower treatment burden [234] and longer duration of effect [192,217,235]. (Figures 2,3)



**Figure 2:** (A) Preoperative red-free fundus photograph of patient with diffuse clinically significant diabetic macular edema and foveal cysts. (Note film development artefacts superior to fovea and at temporal edge of photograph.) (B) Late phase preoperative intravenous fundus fluorescein angiogram of diffuse, clinically significant diabetic macular edema, with cystoid leakage pattern in fovea. (C) Red-free fundus photograph 8 months following 602 confluent grid applications of MRT throughout the macula extending to the edge of the fovea circumferentially.. Note marked reduction in macular edema without visible chorioretinal scarring or pigmentary disturbance. (D) Postoperative intravenous fundus fluorescein angiogram demonstrating marked reduction in diffuse, cystoid leakage, and without angiographically visible pigmentary disturbance or chorioretinal scarring [from: Luttrull, et al, 2005].

Regarding treatment of the progressive peripheral retinal as well as macular capillary non-perfusion and neuronal apoptosis, a small number of studies employing MRT principles have been reported wherein MPL is applied in a contiguous fashion over the macula and peripheral retina [188,233,237]. However, the results mirror the results of MRT for DME, showing efficacy comparable to conventional photocoagulation without adverse treatment effects, and visual results comparable to drug therapy but with a longer duration of action. (Figure 4)



**Figure 3:** Spectral-domain optical coherence tomograms (SD-OCT) of eyes with fovea-involving DME and chart BCVA's better than 20/40 treated with transforeal MRT applied throughout macula. Despite mild macular thickening and good pre-treatment VAs, both macular thickening and VAs were significantly improved following treatment. In each pair, A-D, top of the frames represent preoperative SD-OCT and bottom of the frames represent postoperative SD-OCT). A. Intraforeal cysts without retinal thickening. B. Intraforeal thickening with minimal central foreal thickening. C. Isolated central foreal cyst. D. Diffuse macular thickening including the forea [From: Luttrull, Sinclair, 2014].



**Figure 4:** Ten-minute post injection intravenous fundus fluorescein angiograms of eye with proliferative diabetic retinopathy before (left) and 3 years after (right) a single treatment session of panretinal MRT laser, with regression of neovascularization inferiorly, decreased fluorescein leakage and reversal of background retinopathy severity [From: Chhablani J, et al, 2018].

### Conclusion

In all regards, as discussed above, the treatment of the neurovascular degeneration that occurs and progresses among individuals with diabetes as well as pre-diabetes to cause severe vision loss, even with the optimum glycemic control, remains unsatisfactory. This demands an urgency for defining treatment methods that prevent the devastating vision loss caused by this systemic disorder and the effects upon the retina. Certainly, such treatments must be entertained as early as possible in the course with repeated monitoring guidance and reapplication to achieve these goals.

The primary orientation here-to-fore, however, has been to focus interventions upon treating the late DR complications of macular edema and neovascularization with the continued monitoring via retinal imaging including fundus photographs, IVFA and OCT and functional testing by chart acuity, and Humphrey visual fields performed under photopic conditions. As detailed above, these fail to detect the progression of the neurovascular injury until after significant, widespread microvascular occlusion and inflammatory injury cause neuronal apoptosis, death and atrophy. At these stages, the processes are poorly reversible with diabetes now becoming a major cause of mild, moderate and severe vision loss worldwide. What is absolutely required is the adoption of an attitude toward earlier intervention with methods that stop or preferentially reverse the neurovascular injury processes; however, at such earlier stages such treatments must be non-invasive and easily tolerated by patients, with patient appreciated visual function outcomes.

MRT offers the ability to improve inflammation and visual function in eyes with diabetic retinopathy, with indications that the treatment is able to reverse, not just slow, disease progression and with maintenance of these improvements long-term (with guided retreatment as discussed). Because MRT is without adverse treatment effects, it is the first treatment realistically appropriate for preventive use, at a time when the neurovascular progressive injury process is reversible and treatment is most effective [182], and with demonstration that the neurovascular protective effects are prolonged [187,190,191,193,211,225,233,236,237]. Therefore, early treatment, especially prior to the development of classic DME or significant DR, has been discussed above as it can effectively preclude vision loss in most eyes. Thus efficacy, durability, reliable safety and consequent repeatability of MRT appear to make it the currently only viable, early and preventive intervention.

The question arises as to when to initiate intervention for this focal, progressive injury process. In principle, given the safety of MRT, we have proposed initiating treatment when testing of macular vision under mesopic or low contrast photopic conditions demonstrate loss, or when new methods of retinal imaging demonstrate early focal microvascular occlusion (via new constructs of OCTangio that improve quantitative analysis of focal lesions) or early neuronal apoptosis (e.g. via fluorescent staining of ZNDpA) [169,170] when these processes are still potentially reversible.

The frequency of retreatment is dictated by the nature and severity of the disease process in the individual, with the more severe and rapidly progressive situations requiring more frequent retreatment, as often perhaps as every 3-4 months [182]. As a rule, however, the earlier the intervention is begun, the greater the long-term benefit. Compounding exponentially, the long-term benefits of even a slight slowing of a rapidly progressive disease may result in significantly prolonged retention of excellent visual function. Certainly, however, we must evaluate modalities for treatment of the more severe stages, at least to avoid the prevention of the severe blinding outcomes that currently manifest among so many individuals. As discussed above, this most likely will require careful, multiphasic monitoring and multi-modal therapies.

Finally, the treatments must be associated in all cases with rehabilitation methods that understand the integrated visual field and the visual problems associated with real world task failure from focal defects in order to develop the assistive work-arounds together with adequate instruction and training to achieve patient engagement and task management.

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