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Advantages of *Psammomys obesus* as an Animal Model to Study Diabetic Retinopathy

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Abstract

Psammomys obesus is an animal model of type 2 diabetes, which develops diabetic retinopathy as a result of chronic hyperglycemia after a high caloric diet. Distinctive features of induced diabetes in *P. obesus* are vascular structural abnormalities, elevated ratios of pro- to anti-angiogenic growth factors in the vitreous, blood-retinal barrier breakdown, neural and glial changes. Although many existing diabetic animal models develop ocular complications, retinal lesions frequently observed in diabetic patients such as preretinal neovascularization, retinal detachment and neovascular stages are only rarely observed in these models. Nevertheless, existing animal models are useful because preventing progressive capillary obliteration from occurring in the retina is likely to be a more beneficial therapeutic goal than merely inhibiting neovascularization in an already damaged and ischemic retina. This review highlights recent observations regarding the histological changes seen in blood–retinal barrier breakdown, the alterations of macroglial and neuronal pattern in diabetes, and how these changes lead to vision loss. Although, the *P. obesus* will be a useful model in studies of the pathogenesis and treatment of diabetic retinopathy.

Keywords: Diabetic retinopathy; Animal models; *Psammomys obesus*; Neural damage; Vascular abnormalities

Introduction

Diabetic retinopathy (DR) is considered as a disease of the microvasculature of the retina. The pathophysiology involves the loss of pericytes [1], vascular leakage, retinal angiogenesis [2], and alterations in structure and function of glial cells [3,4]. The progression of microvascular disease has been divided into two stages: an early stage (non-proliferative DR (NPDR), and later stage (proliferative DR (PDR)) [5]. NPDR, currently diagnosed by ophthalmic examination, is based on the presence of retinal vascular abnormalities, including microaneurysms, intraretinal microvascular abnormalities, obliterated capillaries, retinal hemorrhages, edema and exudates. All these signs indicate failure of regional retinal microvascular circulation, which probably results in ischemia. The new vessels are the main factors causing vitreous hemorrhage and a decrease in visual acuity in diabetics. They can also contribute to retinal detachment. Retinal edema is another factor causing visual impairment in diabetes [6], which implies the rupture of the blood-retina barrier and leakage of plasma from small blood vessels. The macula, the central part of the retina responsible for high acuity visual function, is particularly sensitive to this thickening of the retina, leading to vision loss.

Many animal models have been used in research for diabetes mellitus (DM) and its complications. Streptozotocin (STZ) or Alloxan induced DM models are the most widely used [7], but also genetic models such as Nonobese diabetic (NOD) mice [8], Bio-Breeding (BB) rats [9], *ob/ob* mice [10], *db/db* mice [11], Goto-Kakizaki (GK) rats [12], Zucker diabetic fatty (ZDF) rats [13], and Otsuka Long-Evans Tokushima fatty (OLETF) rats [14] are common. Although these animal models develop either type 1 or type 2 diabetes and subsequent ocular complications, the severe retinal lesions frequently observed in human diabetes patients such as preretinal neovascularization or retinal detachment are not found; at most, early pathological changes such as pericyte loss [15,16], retinal leukostasis [17], and abnormal patterning in electroretinograms (ERG) [18] are observed. In these models, the pattern of progression and symptoms closely mimic those of diabetes mellitus in humans and play a significant role in diabetes research, even though any single model may be inadequate for clarifying all the issues related to the disease. We established a new DR animal model, the desert sand rat (*Psammomys obesus*), which is long known to develop metabolic stress syndrome in captivity. In contrast to individuals maintained on a natural plant-rich diet, when reared on a high calorie regimen many animals exhibit hallmark features of type 2 diabetes [19]. The similarities between metabolic, physiological and endocrine changes in this species and those occurring in human type 2 diabetes make it a highly relevant animal model to understand pathogenesis of this disease [20]. Aside from a single study on tyrosine hydroxylase levels during diabetes [21], retinal modifications occurring in this species have not been reported. We demonstrated recently that the sand rat *P. obesus* has a remarkably cone-rich retina [22], as seen in other diurnal rodents, and represents a useful adjunct to available animal models of central vision. As a result of chronic severe hyperglycemia, *P. obesus* develops DR [23]. In addition, severe alterations such as cataract, microaneurysms, loss of pericytes, blood-retinal barrier breakdown and profound alterations in glial and neural cells are seen in *P. obesus* [23]. In the present review, we describe pathophysiology of ocular complications in diabetic *P. obesus* and make a structural comparison with other animal models of DR.

Vascular Changes

Pericyte

Vascular abnormalities in *P. obesus* retina are characterized

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primarily by the loss of pericytes described among the first symptoms of early stages of retinopathy [1,24]. It is considered as one of the initial changes before the onset of vascular lesions in DR, such as the formation of acellular capillaries [1]. Loss of pericytes in the retinal microvasculature contributes to the development of retinopathy and onset of hyperpermeability of blood vessels. Their loss, coupled with endothelial cell apoptosis, seems to lead to the formation of acellular capillaries [1], a phenomenon that could contribute to increased permeability of blood vessels. Loss of pericytes was studied previously in Wistar rats injected with STZ after 3 months of induced diabetes [25]. Similar changes were observed in *P. obesus* after 5 months of induced diabetes [23].

Pericyte migration represents a new mechanism for the loss of pericytes in DR. The mechanism is still unclear but seems to be regulated by signaling along the Ang-2/Tie-2 pathway [26]. Many reports have shown increase in the proportion of endothelial cells/pericytes (E/P) in the retina of patients with diabetes, as well as animal models [24,27], and some studies attributed this change to the loss of pericytes induced by diabetes.

Pericytes, retinal Muller glial cells (RMG) and endothelial cells form the blood-retinal barrier. Pericyte degeneration is one of the earliest pathological changes seen in DR, and this loss disrupts the cellular metabolism of endothelial cells [28,29]. Reasons for pericyte death are thought to include over-activation of the protein kinase C (PKC) pathway, and by increased production of advanced glycation end products (AGEs) [30].

Among the other changes of blood vessels in DR, the presence of acellular capillaries indicates non-functional and degenerated capillaries, and is considered as non-perfused [31]. The blockage of the capillaries observed in humans, *P. obesus* and other animal models, occurs first in diabetes but has no clinical significance. The existence of such acellular capillaries is not enough to signify the presence of DR; however, the pathology is confirmed when the lesions become more important [32].

Structural abnormalities

Another vascular lesion characteristic of DR in humans is microaneurysms. These lesions are not reported in C57BL/6 or Ins2Akita mouse [33,34]. We reported the presence of microaneurysms in diabetic *P. obesus*. In other species such as KK mice, microaneurysms are found in older individuals [35]. Moreover, the study in *db/db* mice has shown an increase in capillaries of the retina in the inner nuclear layer (INL) [36] without extending into the vitreous body.

Pro-angiogenic factors

Vascular endothelial growth factor (VEGF): Western blot analysis showed up-regulation of the pro-angiogenic factor (VEGF), and down-regulation of the anti-angiogenic factor pigment epithelium derived factor (PEDF) in the vitreous of *P. obesus*. VEGF represents an important indicator of neovascularization, which will contribute to both microaneurysms and formation of new retinal blood vessels leading to vascular ischemia and hemorrhages, respectively. In most species studied, the major sources of VEGF are the retinal pigment epithelium (RPE), neuronal cells (especially the retinal ganglion cells (RGC)) and RMG [37]. VEGF and its high affinity receptor are found in the retina and may be important to maintain the homeostatic balance of the vascular tissue. It is known that VEGF increases in the retina of diabetic patients during the early phase of the disease [38]. This factor may be involved in the increase of permeability of retinal blood vessels observed in the preclinical stage of DR [39]. Indeed, since its discovery VEGF is considered the most powerful factor to increase permeability of vascular tissues in diabetes [40]. In addition, VEGF pathways increase the expression of nitric oxide synthase mRNA (NOS) and NO production [41].

To determine the pathogenic mechanisms of micro-vessel disease, Cukiernik and his colleagues examined the role of VEGF and its interaction with other factors in diabetic STZ mice [42]. VEGF may interact with intercellular adhesion molecule (ICAM-1), to increase permeability of blood vessels in diabetes [43].

Another important mechanism leading to an increase of VEGF in diabetes is the activation of the PKC pathway [43,44]. Other routes concern polyols that can also regulate the expression of VEGF, as well as non-enzymatic glycation and oxidative stress [45]. The inhibition of VEGF reduces vessel growth in rodent models (rat, mouse) of cancer, rheumatoid arthritis and eye diseases [46,47]. VEGF causes uncontrolled neovascularization that damages the retina, but also encourages the leakage of blood vessels and hemorrhage that lead to blindness. In human retina, the expression of this factor is elevated in patients with diabetes, as is seen in other models of DR and in *P. obesus* [23].

Pigment epithelium derived factor (PEDF): The concentration of angiogenic vascular factor VEGF is balanced by the synthesis of several anti-angiogenic factors, such as PEDF, angiostatin, endostatin and thrombospondin. In therapy, steroids, monoclonal antibodies, blocking of VEGF receptors, inhibitors of signal transduction and antagonists of the extracellular matrix have all been tested [48].

PEDF is an important inhibitor of proliferation and migration of endothelial cells, it suppresses ischemia caused by neovascularization of retinal blood vessels [49,50]. This anti-angiogenic factor is a glycoprotein of 50 kDa synthesized by RPE, it belongs to the superfamily of inhibitors of neuronal serine protease activity [50]. In human eyes, PEDF is decreased in the vitreous of patients with proliferative DR (PDR) [51]. The same observation was made with our spontaneously diabetic animal model *P. obesus* but not with other animal models of DR such as rats injected with STZ [52], and spontaneously diabetic rat Torii (SDT) [53], which show high levels of PEDF. Low levels of PEDF were associated with angiogenesis of blood vessels, leading to proliferative DR according to Ogata et al. [54]. Therefore, PEDF is considered as a therapeutic target for eye diseases that involve oxidative stress, such as PDR [55]. The balance between pro-angiogenic and anti-angiogenic factors is critical to determine the development of PDR. The study of the ratio of VEGF/PEDF reveals that the PDR has highest ratio in human [56] similar to those obtained in our results in *P. obesus* vitreous (Figure 1) and unlike other DR animal models [53,57,58].

PEDF protects cells such as pericytes and neuronal cells against oxidative damage [59]. PEDF inhibits AGEs and hyperpermeability in the retina and blood vessels *in vitro* [59]. PEDF induces endothelial cell apoptosis in new blood vessels, causing the inhibition of proliferation of these cells [60]. Nonetheless, the inhibitory mechanism action of PEDF on blood vessels, resulting from AGE and retinal endothelial cells apoptosis, remains to be clarified [61].

The different DR studies found in the literature do not clarify whether PEDF has a direct effect on the permeability of blood vessels, or inhibits the production of VEGF. Further investigations are necessary to determine the interaction between PEDF and VEGF in the pathogenesis of DR in diabetic *P. obesus* individuals.

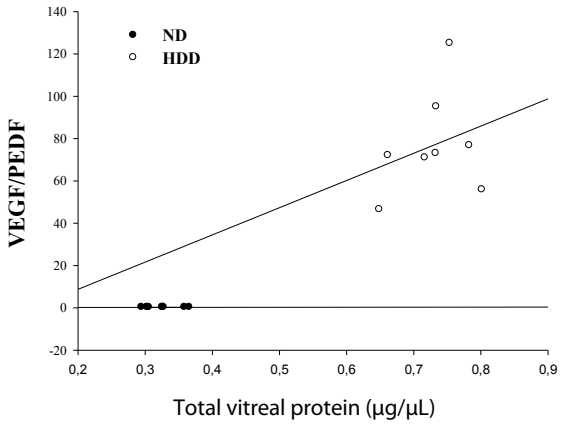

Figure 1: Vitreal Proangiogenic and Antiangiogenic Growth Factor ratio as a function of total vitreal protein content in ND and HDD *P. obesus*. Solid line is the result of correlation (linear) analysis. A statistically significant relationship was found between total vitreal protein content and VEGF/PEDF ratio. The correlation coefficient (r) was 0.23.

Abbreviation: ND: Natural Diet; HDD: High-protein Diet Diabetic [23].

VEGF and PEDF are important factors to maintain the retinal blood barrier. This latter is composed of tight junctions located between the endothelial cells of the retinal vessels and RPE and protects the retina against circulating molecules and cells, to confer immune privilege to the eye, and to limit the penetration of drugs into the retina. RPE cells are responsible for the hydro-ionic exchanges between the choriocapillaries and outer retina [62,63].

Tight junction proteins

Occludin is an important tight junction protein responsible for retinal-blood barrier formation [64]. This tight junction protein is reduced during the first weeks of diabetes in STZ injected mice, in correlation with increased permeability to serum albumin [65], reflecting the relationship between the tight junction protein and permeability of endothelial cells [65]. Similarly, the results *in vivo* have shown a reduction of tight junction proteins associated with the permeability of blood vessels [66].

The immunoreactivity of occludin in normal mice is more intense in blood vessels and arteries, but less intense in the postcapillary venules [67]. This protein is colocalized with zonula-occludin in the RPE of *P. obesus*, but also in the retina (horizontal cells and outer limiting membrane (OLM)) [22]. It can be disrupted in OLM as part of the blood-retinal barrier in pathological conditions, which contribute to fluid accumulation in the macula [63]. These tight junction proteins are altered in diabetic *P. obesus* (Figure 2), mice and monkeys [63].

Blood vessels and glial cells of the retina are in close contact and are able to communicate directly with each other [68]. Diabetes may increase the permeability of blood vessels in the retina and can change components of the optic nerve [69,70], disrupt interactions between neurons, glial and endothelial cells [71]. Therefore, integrity of blood-retinal barrier (vascular permeability degree) depends on factors released by glial cells [72]. Increased expression of tight junction proteins is due to factors secreted by astrocytes. The redistribution of glial fibrillary acidic protein (GFAP) in the astrocytes and RMG of diabetic rats was reflected in changes by occluding in vascular

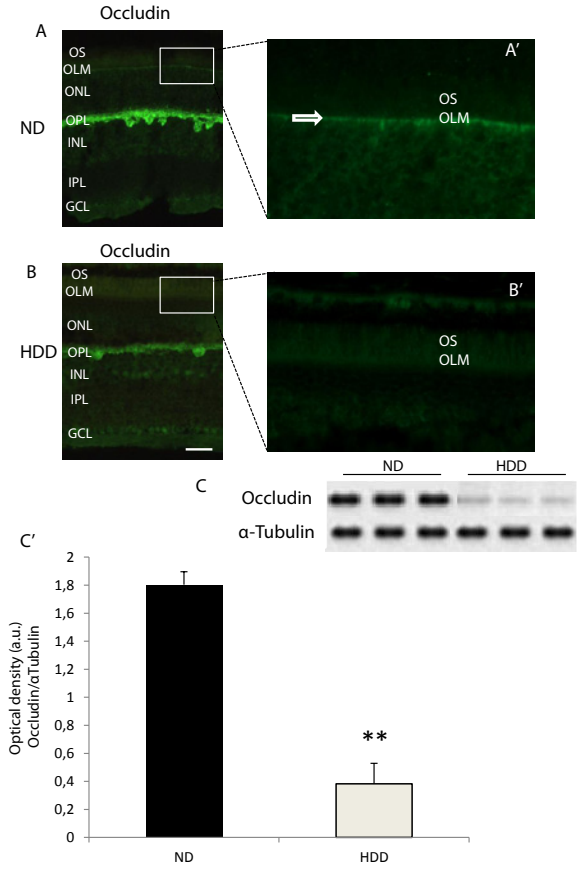

Figure 2: Immunoreactivity of tight junctions protein (Occludin) in *P. obesus* retina. Occludin is expressed as a discrete line at the level of the outer limiting membrane (OLM) in normal *P. obesus* (A, A'), but is almost absent in diabetic animals at this level (B,B'). Quantitative densitometric measurement of band densities compared to α-tubulin confirmed the decreases in HDD (**p<0.01, ANOVA) samples (C, C'). Triplicate blots of occludin and α-tubulin are shown above each corresponding column. Bar in B =50 µm for all panels.

Abbreviation: GCL: Ganglion Cell Layer; INL: Inner Nuclear Layer; IPL: Inner Plexiform Layer; ONL: Outer Nuclear Layer; OPL: Outer Plexiform Layer; OS: Outer Segments; ND: Natural Diet; HDD: High-protein Diet Diabetic.

endothelial cells. Diabetes reduced occludin immunoreactivity in the capillaries and induced redistribution from continuous cell border to interrupted, punctate immunoreactivity in the arterioles. Therefore, the astrocytes increase both vascular endothelial cell barrier function and tight junction protein (Zonula occludens (ZO-1)) synthesis [67,73]. This mechanism is related to the integrity of the blood-retinal barrier *in vivo* [74].

Changes in the Neural Retina

Structural changes

It is acknowledged that structural and functional damage also occurs in non-vascular cells in the retina of diabetic patients [75]. Delayed reduction of latency and amplitude of the oscillatory potential of the ERG have been often seen in diabetic patients [1]. Immunohistochemical analyses of the human retina are of poor quality because of the long post-mortem delays, but the results obtained with other species, especially rodents, show loss of RGC, horizontal, amacrine and photoreceptor cells by apoptosis, a few weeks after the onset of diabetes [76,77]. Diabetes leads to dysfunction and

degeneration of cells by apoptosis in post-mortem of human and animal retina. However, some results in mice are not in agreement with those obtained in rats [78].

Recent studies of retinal thickness in diabetic mice showed a reduction in the layers [33,79]. There was reduced thickness of retinal layers of Ins2Akita mice after 22 weeks of hyperglycemia [33], and in some studies of diabetic C57BL/6 mice injected by STZ [78-80]. Our results show that *P. obesus* retina has a thinned and scalloped appearance after 5 months of diabetes. However, this reduction seems more related to obesity rather than the diabetic state itself, since the differences were also seen in the strain that gains weight without exhibiting hyperglycemia [23]. Spontaneous diabetes induces the loss of RGC of Ins2Akita mice [33]. Five to six months of hyperglycemia leads to a significant decrease in the number of cell bodies of RGC layer, accompanied by a significant decrease in the thickness of the internal plexiform layer. Gastinger et al. have shown that diabetes causes a loss of 16% of RGC in the peripheral retina [81], whereas the central region is not affected. The number of dopaminergic and cholinergic amacrine cells in the retina are decreased in diabetic patients [82]. We noted the loss of cell number at different cell layers in the retina of *P. obesus* including ganlion cells [23]. We reported in this study the increase of immunohistochemical staining of tyrosine hydroxylase in amacrine cells of diabetes animals in comparison with control (Figure 3).

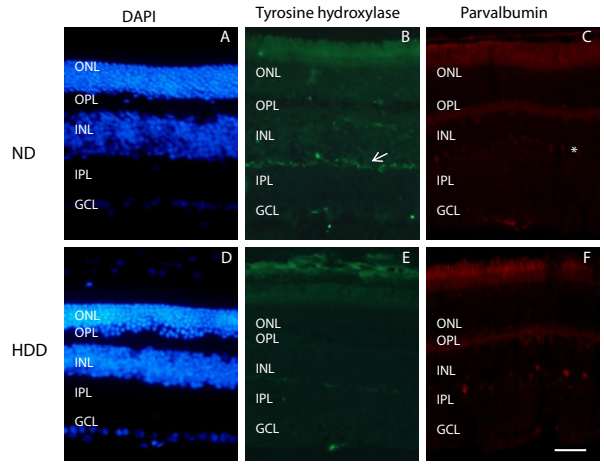

Figure 3: Immunodetection of tyrosine hydroxylase and parvalbumin in the retina of *P. obesus*. Tyrosine hydroxylase immunostaining was intense in plexiform layers in control (B, arrow) but it was reduced in diabetic animals (E, asterisk). Histological sections of *P. obesus* retina immunostained with parvalbumin antibody were very similar in control (C) and diabetic animals (F). Bar in F=50 µm for all panels.

Abbreviation: GCL: Ganglion Cell Layer; INL: Inner Nuclear Layer; IPL: Inner Plexiform Layer; ONL: Outer Nuclear Layer; OPL: Outer Plexiform Layer; OS: Outer Segments; ND: Natural Diet; HDD: High-protein Diet Diabetic.

Ganglion cells

There was a reduction of 20% to 25% of RGC in C57BL/6J mice at 14 weeks of diabetes [83]. In our study, diabetic *P. obesus* retina showed a significant reduction (44%) of RGCs. Other studies in rats and mice have shown no loss of RGC during one year of diabetes [77,78,84,85]. The apoptosis of RGC and other cells of the retina in the *db/db* mice increased [36]. Similarly, after one month of diabetes, the number of apoptic RGC and internal nuclear layer is higher in diabetic KKAy mice compared with the control group, as the rate of cell death increased during diabetes [86].

Many studies of RGC cultured *in vitro* with glucose-rich medium showed an increase of synaptic terminals length. Total density and a number of synaptic terminals and structural reorganization of dendrites were affected [77,87]. These changes are mainly related to the ON-type RGC but not the OFF-type [81]. Similar morphological changes have been observed *in vivo* in human diabetic retina and rats injected with STZ [88,89]. Moreover, the axons of RGC observed by NF200 immunostaining have many varicosities in *P. obesus* and rats injected with STZ [88]. These data indicate that there are morphological changes of retinal RGC subtypes in diabetes condition, revealing the alteration of function of these cells.

Glial cells

Expression of glial fibrillary acidic protein (GFAP): Glial cells are an important element connected directly with retina and retinal blood vessels. Over-expression of GFAP has been often observed in the retina of diabetic rats [3,4], diabetic patients [90], and diabetic *P. obesus* [23]. RMG are associated with astrocytes, endothelial cells and neurons, and play a regulating role in blood-retinal barrier [91]. Under normal conditions, GFAP is expressed only by astrocytes [92]. In ischemic conditions, our results in diabetic *P. obesus* retina have shown over-expression of GFAP by astrocytes and RMG [23]. Barber [93] found that the increased expression of GFAP appears to be produced only in the RMG and preceded by reduced expression in astrocytes, indicating that the sub-type occluding glia has differential response to diabetes [67]. The activation of glial cells is not found in the retina of diabetic C57BL/6 mice [78,84] or in diabetic Ins2Akita mice [33]. Diabetic *db/db* mice and animals without aldose reductase (an enzyme involved in glucose metabolism [36]) have shown an inhibition of GFAP expression in glial cells during diabetes. Structural changes are observed in the microglia of diabetic rat retina by alloxan [85]. The reasons for these changes are not yet known.

The effect of glial activation on capillaries degeneration and neurodegeneration of the retina during diabetes are not well studied. The glial activation and loss of RGC does not seem to occur in all diabetic animal models studied, indicating that changes in retina can be controlled by different ways involved in the diabetic vascular lesions [69].

Expression of glutamate: Other changes in glial cells function suggest that the metabolism of glutamate could be affected in the retina by diabetes. Glutamine synthetase and glutamate aspartate transporters are reduced in diabetic *P. obesus* retina, indicating alterations in glutamate metabolism [23]. Decreased levels or activity of both proteins are observed in other rodent models [94,95]. Elevated levels of glutamate are also detected in the vitreous of diabetic animals [3] and humans [96], indicating disturbed glutamate metabolism.

Many studies have shown that glutamate excitotoxicity is responsible for the loss of neurons in DR [97,98]. Some of them suggest that diabetes increases glutamate level in the vitreous body [3,96]. DR also reduces the ability of the retina to convert glutamate. Diabetes leads to dysfunction and degeneration of cells by apoptosis in post-mortem human and animal retina. [14 C] was converted to glutamine [14 C], presumably due to reduced glutamine synthetase [3]. These data suggest that diabetes disrupts the metabolism of glutamate through two different enzymes. These changes may be preceded by reduction in the activity of glutamate transporter in RMG, which may increase the glutamate concentration in extracellular medium [94].

Amacrine cells

Several studies have demonstrated neuroprotective effects of dopamine against glutamate neurotoxicity [99]. The dopamine production is reduced in the diabetic retina [82]. This decrease is mainly due to a reduction in quantity and/or activity of tyrosine hydroxylase, the enzyme constituting the rate limiting step of dopamine biosynthesis. In addition, the precursor quantity in diabetic retina could be low, leading to reduced tyrosine hydroxylation efficiency. Tyrosine hydroxylase levels are lower than normal in diabetic rat retina [100]. The same result was observed in *P. obesus* retina by Larabi et al. [21]. Our results have shown that the staining of amacrine cells with anti-tyrosine hydroxylase is not intense in diabetic animals in comparison with control animals (Figure 3). Amacrine cells, acting as integrators of signals from cone-bipolar cell to RGC, use parvalbumin as a cytosolic sensor via a Ca^{2+} dependent mechanism [101]. In rat retinas, parvalbumin is found in amacrine cells [102]. A-II type amacrine cells, the most frequent sub-class, are responsible for transmitting signals from RGC to bipolar cells [102]. In *P. obesus* retina we did not observe a difference in the expression of parvalbumin in different groups (Figure 3), despite the several changes of retinal neurons such as cones and bipolar cells [23]. Reduced expression of dopamine by diabetic retinal amacrine cells is linked to that of RGC [103]. The expression of parvalbumin is increased in cone bipolar cells in diabetic rat retina injected by STZ [104].

Bipolar cells

Bipolar cells are also affected by diabetes. Activated bipolar cells express several isoforms of PKC. The localization of α, β, γ, ε and ζ PKC isoforms was shown in the rabbit retina by immunohistochemistry [105]. The sub-types α, β, γ of PKC sensitive to Ca^{2+} are located in different populations of neurons. The isoform ζ, which does not need Ca^{2+} to be activated, is co-localised with PKC-α [105]. Among the different isoforms, PKC-α is the most abundant [106].

The activation of PKC by hyperglycemia can change the action of insulin on blood vessels [107]. There are many reports about the effect of PKC activation on secretion, resistance and action of insulin. Das Evcimen and King have shown that isoforms β II, ε, α and β I are more activated in diabetes *in vitro* [107]. Multiple studies have shown that the activation of atypical ζ isoform of PKC plays an important role in pathological mechanisms of diabetes. Insulin can activate phosphatidylinositol 3-kinase (PI3K), 3-phosphoinositide-dependent kinase-1 (PDK-1), and PKC ζ [108]. The activation of PKC ζ by insulin has a significant impact on protein synthesis [109]. However, many studies suggest the activation of PKC isoforms, indirectly by PI3K, prevents insulin action [110].

Our results in *P. obesus* retina show decreased expression of both PKC α and ζ isoforms in diabetes [23]. These two isoforms are Ca^{2+} independent and co-localised in rod bipolar cells. This decreased expression may be related to photoreceptor alterations in diabetic *P. obesus* retina [23]. Molecular and cellular mechanisms of PKC in DR have not yet been fully clarified. The beta-II isoform of PKC is the most studied among the other isoforms in different animal models of DR [111]. This isoform is over-expressed in the retina of diabetic *P. obesus* (Figure 4), as is the case in the vast majority of animal models of DR [111].

Stimulation of PKC is necessary to activate VEGF [112], which was identified as one of the main mediators of DR [113]. Most studies show that the activation of PKC can reduce blood flow in the retina with less than 10 years of diabetes [114]. After 10 years of diabetes, blood flow

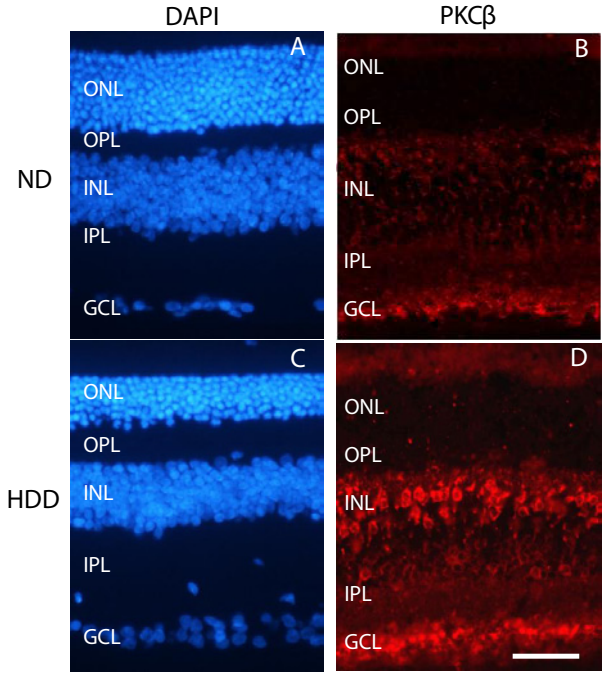

Figure 4: PKCβ immunostaining in normal and diabetic Psammomys retinas. In ND retinas, residual staining with PKCβ antibody was visible in the synapses (B) but in HDD retinas, PKC β antibody showed clear staining in cell bodies within the inner nuclear layer (INL), as well as their axons and synaptic endings in the inner plexiform layer (IPL), corresponding to rod and cone ON bipolar cells D). Bar in D=80 µm for all panels.

Abbreviation: GCL: Ganglion Cell Layer; INL: Inner Nuclear Layer; IPL: Inner Plexiform Layer; ONL: Outer Nuclear Layer; OPL: Outer Plexiform Layer; OS: Outer Segments; ND: Natural Diet; HDD: High-protein Diet Diabetic.

in the retina appears to be increased [115]. Blood flow anomalies and retinal ischemia contribute to vessel dysfunction. Retinal ischemia is the result of increased expression of vascular growth factors such as VEGF, leading to macular edema and PDR [116].

The decrease in PKC phosphorylation can reduce Na+/K+ ATPase phophorylation in vascular and neuronal tissues of diabetic patients and animals [117,118]. It can lead to lowered neuronal conduction and nerve regeneration [119]. The activation and expression of different growth factors by PKC activation [116,120] may indirectly affect capillary permeability. High expression of VEGF and vascular permeability factor (VPF) is seen in diabetic patients and animal retinas, and participates in neovascularization of PDR [116].

Expression of presynaptic proteins

Other alterations were detected in diabetic retina, including the synaptic terminals. Diabetes reduces the expression of presynaptic proteins and reduces basal synapsin phosphorylation in rat retina [121]. We also noted an increase in synaptic proteins such as synaptophysin (SVP 38) in *P. obesus* [23]. Functional disability in rodent's retina may be the result of deficit of specific presynaptic proteins. Studies by VanGuilder et al. [121] showed that the ability of retinal synapses to conduct regulated neurotransmission is considerably reduced by one month of experimental diabetes in the rat. The retinal content of the synaptic proteins synaptophysine, synapsin I, Vesicle-associated membrane protein 2 (VAMP2), Synaptosomal-associated protein 25 (SNAP-25) and Postsynaptic density protein 95 (PSD-95), as well as the basal phosphorylation of synapsin I is reduced within this short period

of diabetes. These changes indicate that diabetes has a wide effect on retinal synapses, and needs early intervention to prevent or reverse the neuronal dysfunction in DR.

Photoreceptor cells

Glucose is necessary for the maintenance and function of all animal cells. For cells that do not require insulin to increase glucose transport, such as photoreceptor cells, increased extracellular glucose concentrations lead to increased intracellular levels by diffusion through the cell membrane. This metabolism can generate several abnormalities, such as increased consumption of oxygen and high concentrations of sorbitol, decreased concentrations of Myo-inositol [122,123]. Phipps et al. [124] have shown that photoreceptor function is altered by two parameters (contrast sensitivity and color sensitivity) in diabetic patients. The amplitude of the current generated in the dark by the photoreceptor and measured by the ERG a-wave was reduced. The decreased current amplitude rate ranged from 16% to 24% after 12 weeks in diabetic rats injected by STZ. The response of photoreceptors was not affected by diabetes, while dark adaptation occurred faster in diabetic animals compared to controls [124-126]. In diabetic patients, changes in photoreceptor sensitivity were shown by the presence of abnormal amplitudes [127].

Mechanisms to explain these effects include the loss of rod photoreceptors [128], and reduced density of Na+/K+ ATPase dependent channels. Diabetes induces changes in Na+/K+ ATPase activity [129,130] and can also reduce the b-wave. Histological and neurodegenerative anomalies of cones represent about 60% and 30% respectively, in zebrafish retina treated with glucose [131]. Similar results were observed in diabetic *P. obesus* retina [23]. This concurs with epidemiological studies in human, in which severity of DR is related to a longer period of diabetes [131,132]. The disturbances of photoreceptors have been reported only in rods but not in cones of some rodent models of DR [2,133,134]. For humans, disordered functional activity of cones (blue or short wavelength sensitive (S) cones) has been shown in several studies in diabetic patients [135,136]. Decreased numbers of S cones were reported in fovea of diabetic post-mortem patients, but rods were not altered [137]. These results are in agreement with those obtained in *P. obesus* [23].

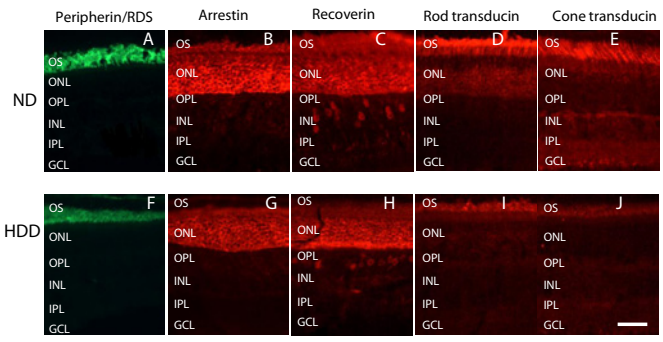

Figure 5: Expression of rod and cone photoreceptor proteins in control (ND) and diabetic (HDD) *P.obesus* retinas. (A,F) peripherin/rds staining is limited to outer segments of both rods and cones; (B,G) arrestin immunoreactivity is strong throughout OS and cell bodies in the outer nuclear layer (ONL); (C,H) recoverin immunostaining is similar to that of arrestin, and also labels scattered bipolar cells in the inner retinal layer (INL); (D,I) rod transducin staining is seen in OS and the lower ONL, corresponding to rod cell bodies (RCB); (E,J) cone transducin is seen in OS and the upper ONL, corresponding to cone cell bodies (CCB). With the exception of recoverin, expression of all proteins was decreased in diabetic conditions. Bar in J=50 µm for all panels.

Abbreviation: GCL: Ganglion Cell Layer; INL: Inner Nuclear Layer; IPL: Inner Plexiform Layer; ONL: Outer Nuclear Layer; OPL: Outer Plexiform Layer; OS: Outer Segments; ND: Natural Diet; HDD: High-protein Diet Diabetic.

We observed loss of photoreceptor cells in the outer nuclear layer (ONL) and a significant decrease of M and S cone opsin expression in diabetic animals [23]. This reduced expression of cone opsins may be related to cell loss, in contrast to rhodopsin expression. Alvarez et al. [131] have observed in zebrafish, an animal model of DR, some morphological alterations in cones. However, studies in nocturnal rodents (rats and mice) have not clearly identified changes mentioned and observed in cones. It can be explained by the scarcity of cones (2-3%) [138]. It would be easier to explore these changes in *P. obesus* retina because it is rich in cones (41%) [22].

The number of cones broadly varies in animal species according to their activity pattern: nocturnal species (rats and mice) of rodents contain only 2 to 3% of cone photoreceptors [138], whereas those diurnal species which have been studied possess 30 to 40% (Arvicanthis ansorgei, Lemniscomys barbarus and Psammomys obesus) [22,139,140]. Kim et al. [125] showed changes in the phototransduction of rat retina in early stage of diabetes. Diabetic animals showed increased expression of rhodopsin kinase (RK) in retina; however expression of transducin (Gαt) and recoverin was decreased. Changes in the RK, transducin and recoverin can induce dysfunctional phototransduction in the early stages of diabetes [141]. In *P. obesus* retina, immunohistochemical studies appear to be insufficient to confirm the results of the expression of these proteins (Gαt and recoverin) obtained in diabetic and control animals (Figure 5).

Recent studies indicate that the responses of RK and Gαt are caused by oxidative stress [67,142] and vascular changes [65]. In addition, the level of Gαt2 declines rapidly in photoreceptors, participating in loss of color sensitivity [143,144]. Changes of RK and Gαt could induce visual dysfunction and vascular abnormalities in blood-retinal-barrier and abnormal phototransduction in diabetes patients [65,67,145-147]. The limited action of RK or Gt on phototransduction has been well studied *in vitro* [148-150]. Regeneration of rhodopsin is sensitive to extracellular glucose concentrations [151], and prevented by oxygen deficiency [152]. A reduction of rhodopsin is observed in diabetes [153] and may explain the concomitant reduction of arrestin. Deactivation of the photo-response begins with phosphorylation of rhodopsin activated by RK, followed by the binding with arrestin [154,155].

Conclusion

In conclusion, *P. obesus* represents a very interesting animal model to study DR. High-calorie diet induces type 2 diabetes very similarly to the condition observed in humans. Some structural and molecular changes observed in *P. obesus* retina have not been observed in other animal models, and appear relatively quickly, showing the advantages of using this animal model compared to others. The cone-rich retina of these animals is an excellent model to study macular responses of the human retina. Finally, it could be used for screening of therapeutic treatments of diabetes complications.

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