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Characterization of a porcine retinal explant demonstrating glucose-dependent changes in the level of factor type 1 alpha

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Diabetic retinopathy (DR) is a leading cause of blindness in the working population of the Western world. Advances in our understanding of specific variables contributing to this condition is restricted by the lack of characterization available with in vitro model systems. In this study we establish a retinal porcine model and investigate the effect of hyperglycemia on the level of hypoxia inducible factor type 1 alpha in the porcine retina.

Porcine retinae were excised from porcine eyes and cultured in 5 or 25 mM D-glucose in Dulbecco's Minimal essential Media (DMEM), without a matrix (2D) or within a matrix (3D) consisting of a co-gel (0.5% agarose plus collagen 0.25mg/ml) in DMEM and maintained in a humidified atmosphere with 5% CO_2 at 37 °C. This process took 30 min. Retinal integrity was monitored by visualization with a light microscope followed by hematoxylin and eosin staining and Gomori Trichrome. Matrix oxygen saturation (% O_2) was recorded with a Microx TX3 oxygen microsensor at time zero and 24 h following incubation. Immunostaining for HIF-1α was performed using primary mouse anti-HIF-1α (1:20) followed by anti-mouse DyLight 488 goat secondary antibody (1:200) and visualized with an inverted fluorescent microscope.

Retinal tissue and vessel integrity were morphologically preserved on 3D matrices versus 2D control evidenced by hematoxylin and eosin staining. A time dependent increase in collagen incorporation within the explant was seen with the Gomori Trichrome stain. Oxygen levels significantly increased within the explant during the 23h test period from 15.5% to 22.3% ($p<0.001$) in media alone and from 10% to 13% ($p<0.05$) with the matrix (n=6) with all values relative to atmospheric pressure. HIF-1α was detected by immune-histochemical analysis in explants at t=0 in 5 and 25mM glucose concentration, with staining of serial sections with DAPi. HIF-1α levels only remained elevated at 24 h in 25 mM D-glucose and was undetected in 5 mM D-glucose.

This study presents a novel model of retinal explants demonstrating that retinal tissue can be cultured in non-hypoxic conditions and that the 3-dimensional matrix was able to sustain retinal structure. Retinal structure was, at least partially sustained by the incorporation of collagen into the retina. Differential expression of protein was identified in the different matrix environment; specifically, hyperglycemia sustained non-hypoxia induced HIF-1α expression in isolated retina.

Diabetic retinopathy (DR) is a microvascular complication of diabetes and is a major cause of visual loss among those aged between 25 and 74 years [1]. Hyperglycemia initiates complex glucotoxicity in the retinal vasculature and surrounding tissue [2,3], leading to changes in the neurovascular network and immune activation [3]. Clinically there is a close association between glucose control and DR as demonstrated by clinical trials for type 1 and type 2 diabetes, with intensive glucose control significantly reducing the incidence and severity of DR [4,5].

Hyperglycemia induces reactive oxygen species (ROS) [6-8], with increased protein kinase C (PKC), activation of aldose reductase, increased hexosamine flux and increased production of advanced glycated end-product (AGE). In addition, endothelial dysfunction in early diabetes is precipitated by acute transient and/or sustained hyperglycemia [9], leading to impaired retinal vessel vasoconstriction, inflammation, and thrombosis. In this late stage, the oxygen-starved environment results in the activation of hypoxia-regulated proteins to ameliorate the deleterious effects of the conditions [10-12].

Hypoxia-inducible factor −1 (HIF-1) is a key protein associated with hypoxia and is a major transcription factor regulating genes, traditionally known to facilitate adaptation and survival of cells in hypoxia (~1% O_2) [10,11]. HIF-1 is a heterodimer complex consisting of a regulated subunit, HIF-1α and a constitutively expressed subunit, HIF-1β, also known as the aryl hydrocarbon nuclear translocator (ARNT) [12]. HIF-1β is constitutively expressed with its mRNA and protein levels maintained at a constant level regardless of oxygen availability. The transcription and synthesis of HIF-1α is constitutive and unaffected in normoxia [12]. However, in normoxia de novo synthesized cytoplasmic

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HIF-1α is degraded with a half-life ($t_{1/2}$) of approximately 5 min resulting in very low levels of HIF-1α protein [12].

Non-oxygen dependent HIF-1α stabilization occurs due to the action on HIF-1α translation, which is different from the classic hypoxic stimuli because hypoxia classically exerts its effect on HIF-1α protein [13]. Once stabilized, HIF-1α translocates to the nucleus where it dimerizes with HIF-1β forming a transcriptionally active complex. HIF-1 signaling is associated with blood-retinal barrier breakdown [12], and it is mediated by long-term physiologic hypoxia in vessels and consequently, hypoxia driven vascular endothelial growth factor (VEGF) over-production which is believed to be is a primary initiator of uncontrolled angiogenesis as characterized in proliferative diabetic retinopathy (PDR) [14].

A range of in vitro models have been used to determine some of these key factors that have been associated with the the onset and progressive pathology of DR [2,3]. Isolated cells have been used to determine some of the key molecular and biochemical parameters that contribute to DR [10-12] and retinal explants have been developed as model systems to look at the interaction of different cell types [15-17]. However, the embedding process of explants may coincidentally create an hypoxic environment for the retina. In this study, a porcine retinal retinal explant model is presented, and the conditions have been determined tto ensure that hypoxia is not compounding any of the results.

The aim is to determine the retinal architecture within the explant, the level of oxygen available to the tissue, to establish the role of a supportive matrix and to evaluate the effect of high glucose concentrations on the level of HIF-1α protein within the retinal explant.

METHODS

Three-dimension matrix: A three-dimensional (3D) co-gel matrix solution was prepared with melted 1% agarose (Bioline, London, UK) and collagen (0.5 mg/ml; Gibco, Paisley, UK) in 5 mM D-glucose Dulbecco's modified essential medium (DMEM; Gibco). Matrix components were mixed to give 0.5% agarose-collagen (0.25 mg /ml; AC) co-gel scaffold (matrix) solution subsequently referred to as the matrix solution. A volume of 1.8ml of matrix solution was added to each well of a 6-well plate, allowed to cool, and solidify. Bed matrices were prepared on the day of each experiment and were kept in an incubator at 37 °C (5% CO_2, 95% air) until required.

Retinal tissue extraction and explant culture: Porcine ocular globes donated from a local abattoir were used for experimental purposes. The globes were removed from the animals within 30 min of slaughter and transferred to the laboratory at 4 °C. Upon arrival the globes were immediately trimmed of muscle and fat tissue, and surface-sterilised using 70% ethanol ensuring that no ethanol penetrated the integrity of the globe. Before the initial inscision was made into the sclera the globes were rinsed in ice-cold 0.1 M phosphate buffered saline (PBS). The retinae were removed as described [15], with the whole retina excised from the base of the optic disc, carefully spread flat on the bed matrix, and overlaid with 1.8 ml matrix solution; cooled to 37 °C and allowed to solidify in a 5% CO_2 37 °C incubator forming a sandwich (henceforth referred to as a retinal explant). In this orientation, the inner and outer retina is in immediate contact with the biomechanical support provided by the matrix. Explants were maintained in pre-warmed serum-free 5 mM or 25 mM D-glucose DMEM media, at 37 °C in 5% CO_2. For comparative purposes isolated retinae were cultured in 6-well plates (two-dimension, 2D) without matrices (control). Experiments were set up in triplicate and repeated on ≥ three independent occasions. The preparative stage of the retina was of 30 min duration and this start point is referred to as time zero (t=0).

Oxygen content of matrix: The percentage of oxygen saturation (O_2%) in the matrix was determined using a Microx TX3 fiber optic oxygen microsensor (140-µm diameter tip-PSt1. PreSens, Regensburg, Germany). The oxygen electrode was positioned using a micromanipulator (Narishige, Japan) and measurements (30s) taken at 4 mm depth at 30 min and 24 h. Media oxygen levels were obtained by inserting the oxygen electrode directly into the media above the matrix and served as a control. Results represent mean % O2 (±SD) calculated after measurement of oxygen levels for 30s on six independent occasions with different retina (n=6).

Visual inspection: The 2-D and 3-D retinal tissues were recorded for up to 72 h with a light microscope (Leica DMIL, Manchester, UK) at 200X on more than 15 independent occasions.

Sample harvest, fixation, and cryo-sectioning: Retinal explants were washed with PBS three times (3X) for 5 min on gentle rotation, fixed with 10% neutral buffered formalin (NBF; Sigma-Aldrich, Dorset, UK) and incubated overnight at 4 °C. Holes were made through the matrix bilayer with a 25-gauge needle without disrupting the retinal tissue to facilitate penetration of the fixative. The bed matrix was removed, and explants were washed 3× in PBS with sections of the explants embedded with an optimal cutting temperature (OCT) medium (Tissue-Tek, Labtech, UK) and cryosectioned into 20 µm thick serial sections on Superfrost Plus and Colorfrost slides (ThermoFisher Scientific, UK), air-dried and stored at −20 °C until use.

Histology: Serial sections were thawed at room temperature and stained with 0.1% Mayer hematoxylin (Sigma-Aldrich) for 10 min, rinsed for 5mins in distilled water and dipped briefly in 0.5% Eosin (Sigma-Aldrich). To identify collagen uptake into retinal tissues, serial sections were fixed for 1 h in Bouin's fixative (5%/9%/0.9% acetic acid/formaldehyde/picric acid; Sigma Aldrich) at 56 °C. Modified Gomori trichrome stain was made in-house; (0.6% Chromotrope 2R/ 0.3% Fast green FCF/ 0.8% phosphotungstic acid with glacial acetic acid (National Diagnostics, South Fulton, GA) in distilled water) [18]. Specimens were washed thoroughly to remove the yellow Bouin's stain and incubated with Modified Gomori (Green) trichrome solution for 20 min, rinsed and differentiated in 0.5% acetic acid for 2 min. Specimens were rinsed in water and mounted with Histomount (National Diagnostics). The retinal morphology was visualized with a light microscope/camera (Leica DM4000B/DFC300-FX) at 200X. Three fields of view per section were obtained and experiments were repeated on more than nine independent occasions

Immunofluorescence: Non-specific staining on serial sections was blocked in 10% normal serum (goat) with 1% BSA (BSA; Santa Cruz, Heidelberg, Germany) in Tris buffered saline (TBS) for 2 h at room temperature (RT). Specimens were incubated overnight at 4 °C with 1:20 HIF-1α mouse monoclonal primary antibody (MA-516, ThermoFisher Scientific). Specimens were washed gently three times (3X) for 5mins in TBS. Non-specific endogenous peroxidase was blocked with 0.3% H_2O_2 in TBS for 15 min at room temperature. Primary anti-HIF-1α was detected by incubating with fluorescently labelled DyLight 488 Goat secondary antibody (1:200; ThermoFisher Scientific) for 1 h at RT. Slides were mounted with water soluble Histomount (Vector Labs, Kirtlington, Oxfordshire, UK) and visualized with an inverted fluorescent microscope (Leica DMIL) at 400X magnification. Three fields of view per target were recorded and experiments were repeated on at least three independent occasions. Staining with DAPI (4′,6-diamidino-2-phenylindole was performed on serial section to determine the distribution of DNA in relation to HIF1α staining.

Data analysis: Data obtained for the oxygen content of the matrix was determined to be following a normal distribution using the Kolmogorov–Smirnov test. The Student *t* test was used for statistical comparison of means. A significant difference was taken at $p<0.05$ (*) or $p<0.001$ (***) with data expressed as mean ± SD.

RESULTS

Visualization of retinal explants: Tissue degeneration was evident in 2D isolated retinal culture within 24 h as visualized with the light microscope (Figure 1) while the matrix embedded samples showed evidence of intact vessel structures throughout the tissue. H&E staining of retina with no matrix revealed a disordered retina with no evidence of intact tissue. In contrast, H&E staining of the matrix embedded retinal samples showed inner and outer nuclear layers, nuclear bodies, and structures such as vascular networks (stained red), with erythrocytes (red) at 30 min (A) and 24 h (B; Figure 2). The Gomori Trichrome stain identified nuclei (red/purple), collagen (green) and erythrocytes/mitochondrion (red) at 30 min, 4 h and 24 h. Collagen was localized in the outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL) and increased in intensity with increasing time in culture (Figure 3).

Matrix oxygen saturation: Oxygen saturation (% O_2) was recorded at the start of the experiment and at 24 h within the media alone samples, and within the matrix at a depth of 4 mm (n=8). A significant increase in the level of oxygen was evident after 24 h for both the media alone sample (15.5±3.8% rising to 22.3±2.6% after 24 h $p<0.001$), and for the matrix sample a smaller, but significant rise was observed (10±4.6% rising to 13±6.1% after 24 h, $p<0.05$; Figure 4).

HIF-1α expression: HIF-1α was detected in explants in normoglycemic (5 mM) and hyperglycaemic (25 mM) conditions 30 min post culture (n=4; Figure 5A). HIF-1α protein was undetected in explant specimens in 5 mM at 24 h (Figure 5B, left). The signal intensity for HIF-1α remained elevated above basal levels at 24 h in explants in 25 mM glucose (Figure 5B, right).

DISCUSSION

The porcine retina bears close resemblance in size, basic retinal structure, and vasculature to the human retina [19] and was therefore selected as the source material for this study. Early biochemical and molecular changes in the retina in response to changes in glucose concentration are of interest for the study of diabetic retinopathy and for the consequent development of targets for therapeutic options that may be effective before the onset of clinical signs of disease.

The inter-relationship between different types of retinal cells has been shown to be important in understanding neurovascular damage induced by a high glucose concentration [13]. The explant offers a useful method of incorporating multiple cell types within culture offering a platform for in situ detection of specific proteins [16]. Similar studies using

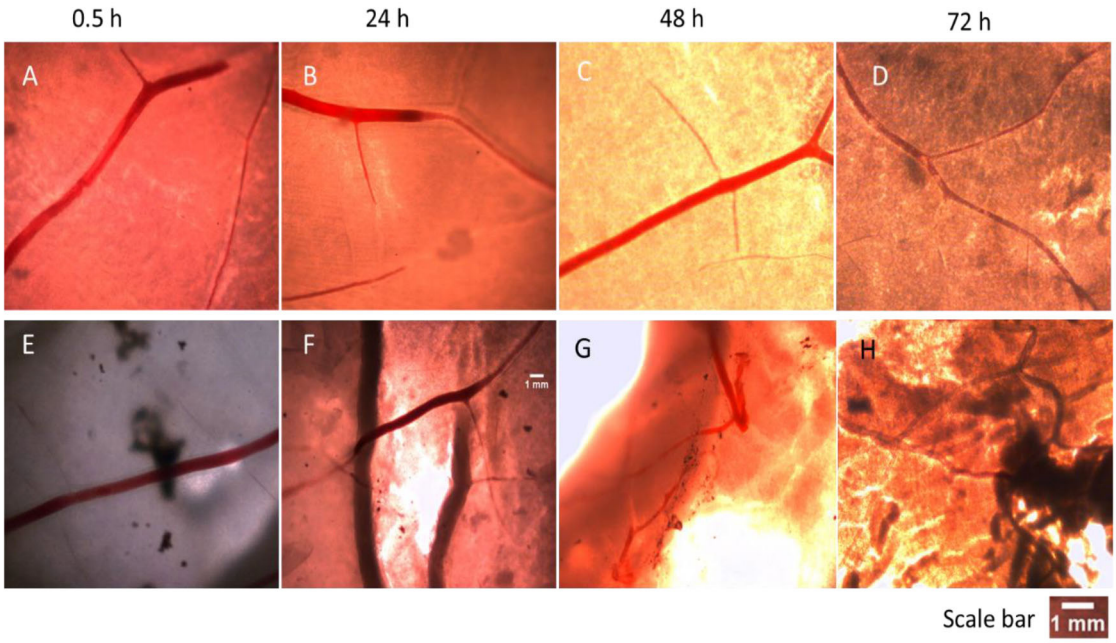

Figure 1. Light microscopic images of explants and 2D control. Explant tissues and vessels showed vessel intactness and absence of degradation for up to 72 h (**A-D**), there are signs of the beginning of tissue tear at 72 h (black arrowhead). Tissue and vessel degeneration was massively evident after 24 h of culture in 2D plates and exaggerated with longer incubation (Black arrows; **E-H**).

small sections of isolated retinal tissue placed on cell culture inserts [17], have been used but in our study the retina is placed within a collagen matrix. It was therefore essential to determine if the matrix was permeable to oxygen and if the retina was able to respond differentially to the content of the matrix which in this case was the high glucose concentration of the matrix.

The critical need for biomechanical support for cell survival within isolated retinal culture is clearly demonstrated in this study. Results for the unsupported retina contrasts with the 3-D support provided by the collagen matrix with respect to tissue integrity. Three-dimensional (3D) culture of explanted retina has been undertaken by several workers, and it is evident that vascular explants are anchorage dependent

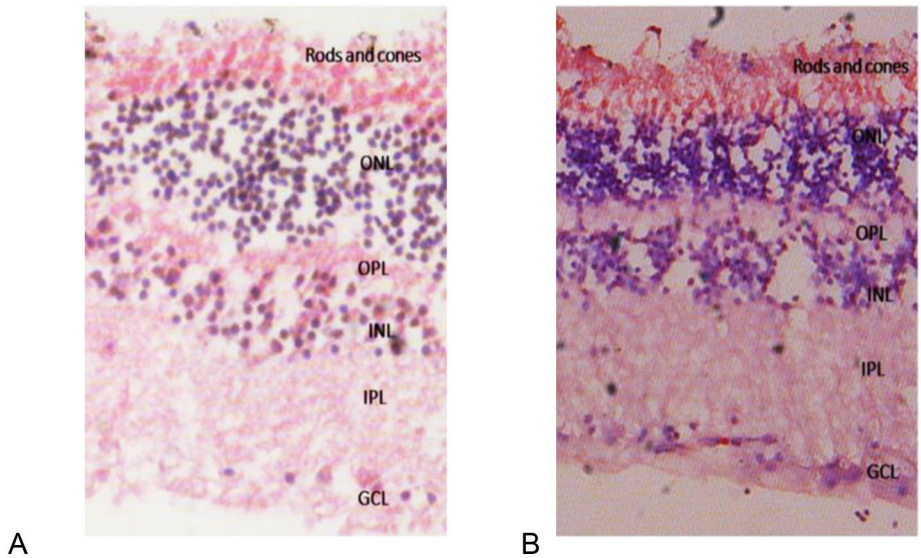

Figure 2. Haematoxylin and eosin staining of retinal explants after 24 h incubation Selected micrographs of retinal explant at t=0 (40X) and 24 h (20X) post culture. Isolated retinae cultured as explants have identifiable retinal layers; photoreceptors, outer nuclear layer (ONL), outer plexiform layer (OPL), Inner nuclear layer (INL), Inner plexiform layer (IPL) and ganglion cell layer (GCL).

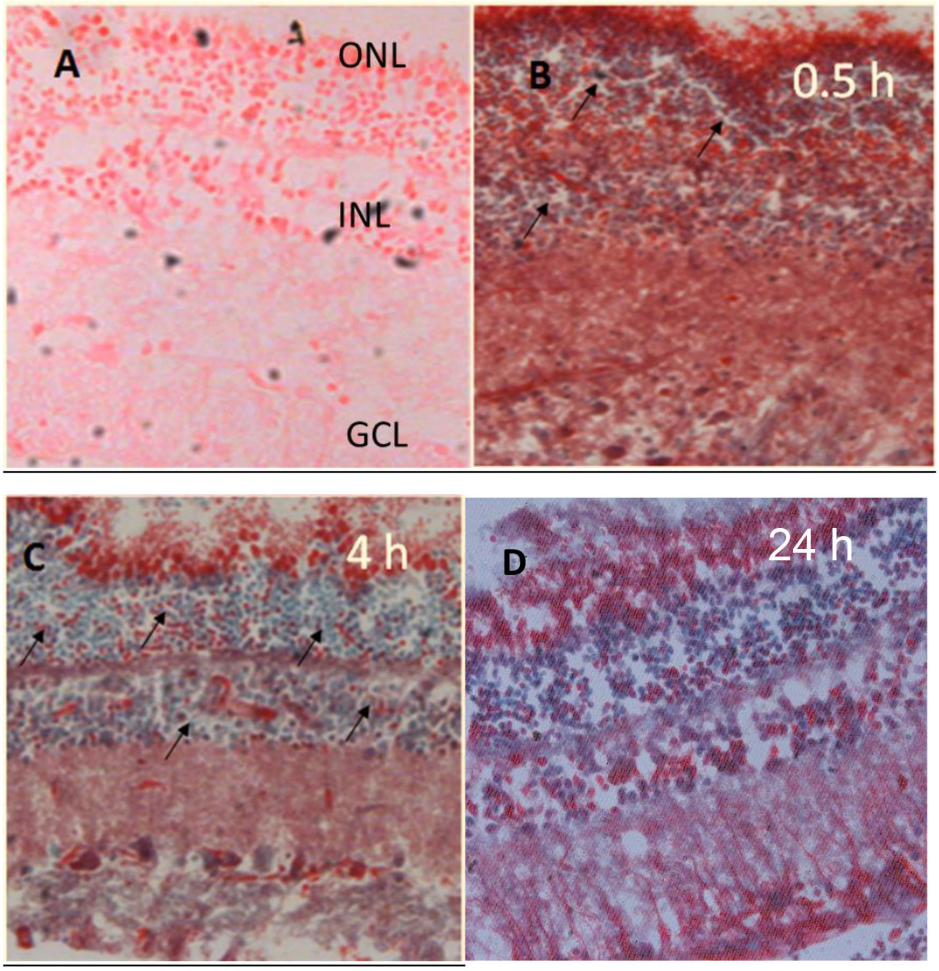

Figure 3. Gomori stain showing collagen within explant tissue. Selected micrograph of retinal explant sections at t=0 (**A** and **B**, 200X), 4 h (C, 200X) and 24 h (D, 400X). A,control micrograph stained with eosin only), **B** (t=0.5 h), **C** and **D** stained Gomori stain and counterstained with eosin. Eosin stain reveals cytoplasmic detail while Gomori stain reveals bluish green collagen. Pink/red stain indicates erythrocytes and cytoplasmic contents of cells. Sections were not counterstained with hematoxylin in order not to mask any positivity for collagen.

[20,21]. Collagen was increasingly evident within the retinal explant sections with increasing time. The collagen may be from one of two possible sources; it may be that the collagen from within the matrix is binding to the surface of cells facilitating maintenance of tissue integrity. Alternatively, the collagen may be produced by the cells within the tissue, as the preparation process of the explant would be a trigger to activate the fibrotic process [22]. Further study with alternate forms of matrix content would help to elucidate the mechanism of the observed response.

A functioning retina has a high metabolic activity as reflected by its origin as an embryological outgrowth of the brain [23]. Oxygen level and glucose-mediated effects are therefore important criteria to understand within an in vitro retinal model. The hypoxic retina responds with the activation of hypoxia regulated genes that lead to an enhanced neovascular response that characterizes the later stages of diabetic retinopathy, namely proliferative diabetic retinopathy (PDR). Here we demonstrate that oxygen was able to permeate the matrix to the level occupied by the retinal tissue within this model reaching 13% from a baseline of 10% indicating that oxygen was able to permeate the matrix during incubation and that initial oxygen concentrations were not hypoxic. Oxygen is reduced in the retina following slaughter and this is evident in the time zero samples where high levels of HIF1a are seen (Figure 5). It is likely that the cessation of systemic blood circulation and inflammation, post sacrifice hypoxia and cellular injury during sample preparation contributed to HIF-1α protein stabilization and detection. Conceivably, metabolically active cells such as the photoreceptors and ganglion cell layer would be particularly prone to such an acute reactive response [24]. Using this model, we were then able to determine a differential effect within the samples based on the concentration of glucose within the matrix. This indicates a specific response of HIF-1α to the concentration of glucose.

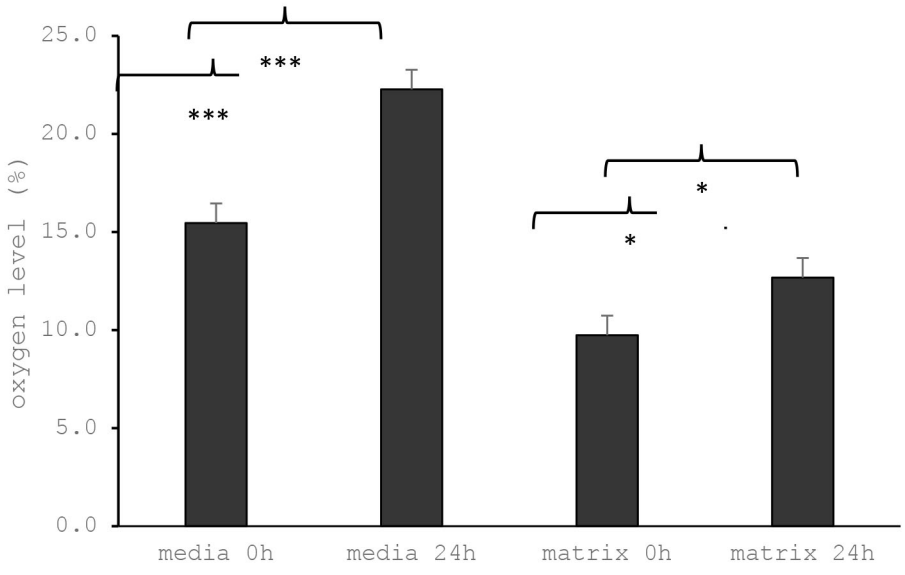

Figure 4. Determination of oxygen content within retinal explants. Mean oxygen saturation increased from time zero to 24 h in media alone (control), from 15.5% ± 3.8 and 22.3%± 2.6, ($p<0.001$) and in the matrices from 10% ± 4.6 to 13% ± 6.1 respectively ($p<0.05$; n=6).

Failure to detect HIF-1α after 24 h in 5 mM glucose conditions is indicative of an increased oxygenation provided by the explant environment and is consistent with the degradation of de novo synthesized cytoplasmic HIF-1α in normoxia to undetectable levels [10], likely via the activation of prolyl hydroxylases (PHDs) [25]. In contrast, the explants exposed to high concentrations of glucose showed, sustained levels of HIF-1α demonstrating a glucose concentration dependent influence on HIF-1α protein levels. HIF-1α elevation (increased translation or decreased destabilization) may

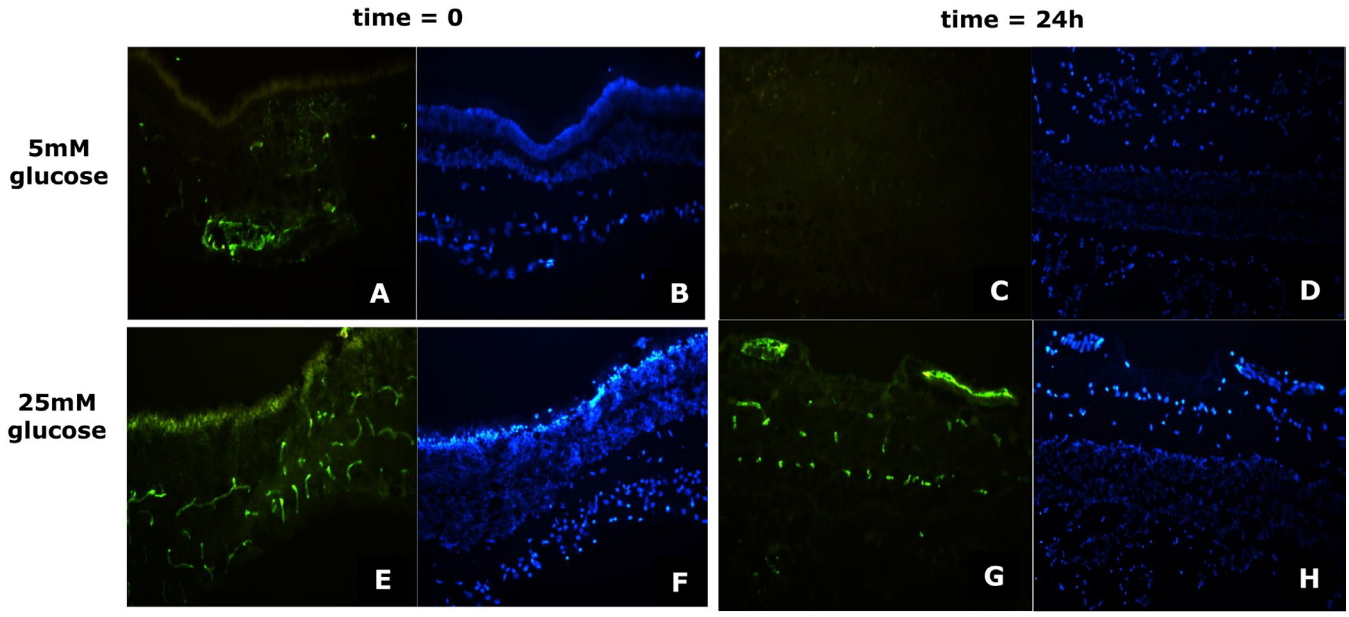

Figure 5. Immunohisotochemical detection of-HIF-1α after 24 h incubation in 5 or 25 mM glucose. HIF-1α protein detection at time zero and 24 h (**B**) exposed to 5 mM or 25 mM HIF-1α protein was detected at in the start of the incubation period in the explants irrespective of glucose concentration. HIF-1α protein levels returned to undetectable basal levels in explants in 5 mM glucose at 24 h but remained elevated in explants in 25 mM at 24 h (400X).

be increasing due to high glucose concentration inducing ROS and inflammatory stimuli in retinal explants [13], rather than a classical hypoxia-mediated stabilization. However, this may also be due to a hypertonic effect due to the elevated glucose concentration [26] and not the result of any increase in glucose metabolism, and further study would be required to exclude this as a possibility.

The role of changes in HIF-1α protein is complex, but it is an important target in the cascade of events that leads to retinal damage, as shown with in-vivo studies using Sprague–Dawley (SD) rats. HIF-1α is correlated with early DR progression and pathogenesis [3] while other studies have demonstrated that increased glucose concentration increases HIF-1α level in human retinal pigmented epithelial cells [13]. However, sustained hyperglycemia induced HIF-1α elevation in isolated retina is deemed noteworthy, especially in conjunction with in-vivo reports of HIF-1α correlation with early DR progression and pathogenesis [27].

Other studies have shown a decreased level of HIF-1α in hyperglycaemic conditions in ulcerated diabetic foot biopsies [28]. HIF-1α destabilization, proteasomal degradation, HIF-1α transactivation inhibition in hyperglycemia under normoxia in endothelial cell lineages of dermal origin is well reported [13]. Cell and tissue type, and species-specific variation will contribute to some of the differences that are reported. It is not possible to determine if the increased level of protein in the retinal explants cultured within an environment of high glucose concentration is due to increased stability of the protein or due to a change in the rate of transcription. Further studies using the explant model would be able to expand out understanding of these events.

Duration of exposure and any consequent changes of the retina due to chronic and fluctuating conditions are also relevant and this is hard to represent in an in vitro model. The short-term effects of glucose concentration in a fully perfused retina are going to be very different to the effect when the retina is damaged with increased vessel permeability, microaneurysms leading to retinal hypoxia and the role of HIF-1α in these conditions will conceivably be different, although no less important. Furthermore, a range of glucose concentrations could be used but the selected concentration of 25 mM had been used previously to demonstrate glucose-specific changes to endothelial cells in the absence of a toxic effect and so this was selected as the concentration of choice [29].

Organotypic cultures of explanted retinal tissue preserves the whole retinal architecture and vessel networks and provides an advantageous environment for cell examination in the context of relevant cell-cell interactions with resident cell types. This study offers a viable model to investigate the influence of a range of conditions on the expression of key proteins, and to determine the effect of potential therapeutic agents. The identification of the expression of key proteins by specific cell types is also possible and would contribute to our understanding of the mechanism of disease and to the potential efficacy of therapeutic agents.

Several factors should be considered before embarking on the use of this model. There is a high level of precision required regarding the initial set up of the explants. Any lack of uniformity of the matrix components and/or the level of the matrix will result in distortion of the resulting tissue sections. In addition, the number of replicate sections from each sample will be limited so care should be taken in the number of variables to be tested. The model did provide consistency in the level of variability between the samples in some aspects of the model set up for example in terms of the level of HIF1α protein. However, there was a high level of variability in retinal vessel responsiveness to KCl and angiotensin II when testing for retinal vessel function. These results are not presented but show a wide range of responses in terms of a reduction or an increase in vessel diameter demonstrating that the vessels are viable and able to respond but that the high level of variability does not allow for meaningful responses to varied test conditions on these parameters.

Conclusion: Diabetic retinopathy (DR) is a microvascular complication of diabetes, and it is a leading cause of blindness world-wide. High glucose concentration induced HIF-1α protein elevation in isolated retina is a potential route of HIF-1α protein involvement in early DR progression and pathogenesis and represents a potential target for therapeutic intervention. Importantly, our model demonstrated the effect of high glucose concentration on the isolated retina in the absence of hypoxia and represents an ideal model for further study.

REFERENCES

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018; 138:271-81. [PMID: 29496507].

2. Brownlee M, Aiello LP, Cooper ME, Vinik AI, Plutzky J, Boulton AJM. 13th ed. 2016. Chapter 33 - Complications of Diabetes Mellitus, William's textbook of endocrinology, pp. 1484–1581.

3. Roy S, Kern TS, Song B, Stuebe C. Mechanistic Insights into Pathological Changes in the Diabetic Retina: Implications for Targeting Diabetic Retinopathy. Am J Pathol 2017; 187:9-19. .

4. Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C. Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993; 329:977-86. [PMID: 8366922].

5. . UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998; 352:837-53. [PMID: 9742976].

6. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000; 404:787-90. [PMID: 10783895].

7. Cameron NE, Cotter MA. Effects of antioxidants on nerve and vascular dysfunction in experimental diabetes. Diabetes Res Clin Pract 1999; 45:137-46. [PMID: 10588366].

8. Ung L, Pattamatta U, Carnt N, Wilkinson-Berka JL, Liew G, White AJR. Oxidative stress and reactive oxygen species: a review of their role in ocular disease. Clin Sci (Lond) 2017; 131:2865-83. [PMID: 29203723].

9. Ceriello A, Esposito K, Ihnat M, Thorpe J, Giugliano D. Effect of acute hyperglycemia, long-term glycaemic control and insulin on endothelial dysfunction and inflammation in Type 1 diabetic patients with different characteristics. Diabet Med 2010; 27:911-7. [PMID: 20653749].

10. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995; 92:5510-4. [PMID: 7539918].

11. Semenza GL. Hypoxia-inducible factor 1: master regulator of O2 homeostasis. Curr Opin Genet Dev 1998; 8:588-94. [PMID: 9794818].

12. Kallio PJ, Pongratz I, Gradin K, McGuire J, Poellinger L. Activation of hypoxia-inducible factor 1alpha: posttranscriptional regulation and conformational change by recruitment of the Arnt transcription factor. Proc Natl Acad Sci U S A 1997; 94:5667-72. [PMID: 9159130].

13. Xiao H, Gu Z, Wang G, Zhao T. The possible mechanisms underlying the impairment of HIF-1α pathway signaling in hyperglycemia and the beneficial effects of certain therapies. Int J Med Sci 2013; 10:1412-21. [PMID: 23983604].

14. Catrina S-B, Zheng X. Hypoxia and hypoxia-inducible factors in diabetes and its complications. Diabetologia 2021; 64:709-16. [PMID: 33496820].

15. Johnson TV, Martin KR. Development and characterization of an adult retinal explant organotypic tissue culture system as an in vitro intraocular stem cell transplantation model. Invest Ophthalmol Vis Sci 2008; 49:3503-12. [PMID: 18408186].

16. Gomori G. A rapid one-step trichrome stain. Am J Clin Pathol 1950; 20:661-4. [PMID: 15432364].

17. Sanchez I, Martin R, Ussa F, Fernandez-Bueno I. The parameters of the porcine eyeball. Graefes Arch Clin Exp Ophthalmol 2011; 249:475-82. [PMID: 21287191].

18. Knott RM, Robertson M, Muckersie E, Folefac VA, Fairhurst FE, Wileman SM, Forrester JV. A model system for the study of human retinal angiogenesis: activation of monocytes and endothelial cells and the association with the expression of the monocarboxylate transporter type 1 (MCT-1). Diabetologia 1999; 42:870-7. [PMID: 10440131].

19. Rettinger CL, Wang H-C. Current Advancements in the Development and Characterization of Full-Thickness Adult Neuroretina Organotypic Culture Systems. Cells Tissues Organs 2018; 206:119-32. [PMID: 30879015].

20. Lai AKW, Lo ACY. Animal Models of DR: Summary and Comparison. J Diabetes Res 2013; 10:106594[PMID: 24286086].

21. Taylor L, Arnér K, Taylor IH, Ghosh F. Feet on the ground: Physical support of the inner retina is a strong determinant for cell survival and structural preservation in vitro. Invest Ophthalmol Vis Sci 2014; 55:2200-13. [PMID: 24595389].

22. Roy S, Amin S, Roy S. Retinal fibrosis in diabetic retinopathy. Exp Eye Res 2016; 142:71-5. [PMID: 26675403].

23. Dowling JE. The Retina: An approachable part of the brain. Part 747. 1st ed. Harvard University Press; 1987.

24. Wong-Riley MTT. Energy metabolism of the visual system. Eye Brain 2010; 2:99-116. [PMID: 23226947].

25. Huang J, Zhao Q, Mooney SM, Lee FS. Sequence determinants in hypoxia-inducible factor-1α for hydroxylation by the prolyl hydroxylases PHD1, PHD2, and PHD3. J Biol Chem 2002; 277:39792-800. [PMID: 12181324].

26. Catrina SB, Okamoto K, Pereira T, Brismar K, Poellinger L. Hyperglycemia regulates hypoxia-inducible factor-1alpha protein stability and function. Diabetes 2004; 53:3226-32. [PMID: 15561954].

27. Thangarajah H. 1, Ivan N Vial, Raymon H Grogan, Dachun Yao, Yubin Shi, Michael Januszyk, Robert D Galiano, Edward I Chang, Michael G Galvez, Jason P Glotzbach, Victor W Wong, Michael Brownlee, Geoffrey C Gurtner. HIF-1α dysfunction in diabetes. Cell Cycle 2010; 1:75-9. .

28. Kelly BD, Hackett SF, Hirota K, Oshima Y, Cai Z, Berg-Dixon S, Rowan A, Yan Z, Campochiaro PA, Semenza GL. Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. Circ Res 2003; 93:1074-81. [PMID: 14576200].

29. Shi H, Carion TW, Jiang Y, Steinle JJ. E Berger VIP protects human retinal microvascular endothelial cells against high glucose-induced increases in TNF-α and enhances RvD1. In, Prostaglandins & Other Lipid Media 2016; 123:28–32.