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Exercise and the diabetic retina: a narrative review

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I, Adam Lamont, declare that the work submitted is my own and is not similar in content to, or based on the work of others, whether published or unpublished, nor does it include contribution of any AI technologies except with full and proper acknowledgement.

Scientific Abstract

The most prevalent microvascular complication of diabetes mellitus (DM) is diabetic retinopathy (DR), which may cause damage to the retina, threatening vision and leading to blindness. Chronic hyperglycaemia has been identified to have a significant role in the pathogenesis of DR. Hyperglycaemia accelerates the inner blood-retinal barrier (iBRB) breakdown through pathways involving oxidative stress (OS), apoptosis, inflammation, and vascular endothelial growth factor (VEGF). This study aims to explore the effects that exercise has on the diabetic retina focusing on pre-clinical animal models.

PUBMED and Ovid MEDLINE were used for data collection from November 2023 to February 2024. A total of 16 searches were carried out to capture all articles relevant to the aims of the review. Studies were included in the review if they were published in English and relevant to diabetes, exercise, animal models or the retina.

A total of 344 results were obtained from the search. 338 articles were excluded due to being duplicates, inaccessible, not in English and unrelated to diabetes, exercise, the retina, or animal models. This review therefore contained 6 articles. 3 common themes from the included articles involved OS, apoptosis, VEGF, and chronic inflammation and the effects of exercise on these was explored.

Analysing results from the papers suggested exercise has beneficial effects on retinal OS, apoptosis, VEGF expression, and the pro and anti-inflammatory profile. Further studies will be required on longer term effects of exercise on the diabetic retina, which could discover potential therapeutic targets for human trials on DR.

Lay Abstract

Long term elevated blood glucose levels due to DM has been related to the progressive damage to blood vessels in the body's vasculature. In DR, damage to the retina occurs when high blood glucose causes changes within the small blood vessels that supply the retina. These blood vessel changes within the retina increases the resistance for blood to flow within these vessels, causing blockages and the vessels to leak. In response there is the formation of new abnormal vessels, which can easily bleed and leak. DR is the most prevalent microvascular complication caused by DM. Prolonged microvascular damage may lead to reduced vision and even vision loss. Currently, there are available treatments for DR, however, they are invasive and expensive. Exercise has proved useful as a therapeutic treatment of diabetic related microvascular complications within the kidney and cardiovascular system. Although exercise has proven beneficial elsewhere in the body in diabetes, previous studies carried out using exercise as a treatment for DR have been inconclusive and results are inconsistent. The aim of this review is to focus on the effects of exercise on the diabetic retina in animal models, to gain understanding on exercises effects on DR mechanisms.

A database search from November 2023 until February 2024 was carried out to capture all relevant articles related to the aims of the project. Key words involved in the database search included; exercise, physical activity, DR, animal*, animal models, diabet*, retin* and vasculature. Papers included within the review had to meet a strict inclusion criteria and papers that failed to meet these criteria were excluded.

6 studies were included in the review from a total of 344 articles which the database search provided. The 338 studies that were excluded did not meet the specific criteria required for the review. Commonly amongst all papers included in the review, exercise had a beneficial effect on the molecules measured within each paper.

By exercise reducing the expression of disease marker molecules, the potential benefits exercise provides may be through the treatment of the pathophysiological mechanisms of DR. The review suggests a potential therapeutic use for exercise in treating patients with DR, however, future studies are required to prove this.

Introduction

DR is a progressive condition and is the most prevalent microvascular complication of DM (1). DR may lead to vision threatening impairment to the retina, which could ultimately result in blindness, being the most common cause of vision loss in adults aged 20-74 (2,3). Almost all type 1 diabetic (T1D) patients and more than 60% of patients with type 2 diabetes (T2D) will develop DR, with approximately 20-30% of patients advancing to the blinding stage (4,5). As the prevalence of DR exponentially rises globally reaching epidemic proportions, individuals with DR are estimated to increase by at least 20% within the next 20 years (6,7). Statistics like this are worrying, placing a growing pressure on global healthcare. Currently, interventions to reduce vision loss involve the prevention of microvascular complications and the treatment of established disease (8). Primary prevention involves the maintenance of blood glucose levels, lipid profiles and blood pressure, as there are no current treatments for early-stage DR (8). For sight threatening DR, treatments are available including laser photocoagulation and intravitreal injections of anti-vascular endothelial growth factor (VEGF) or corticosteroids. These current treatments are invasive and have been associated with increasing intra-ocular pressure, cataracts, endophthalmitis and they are expensive (8).

Blood supply to the retina is rich is characterised by a high level of oxygen extraction and receives its blood supply from both retinal and choroidal blood flow (BF) (9). The short posterior ciliary arteries (SPCA) form arterioles in the choroid. These arterioles branch forming terminal arterioles supplying the layer of the choriocapillaris, outer sections of photoreceptors and retinal pigmented epithelium layer (10). The central retinal artery (CRA) is a branch of the ophthalmic artery supplying the superficial vascular complex (SVC) and the

deep vascular complex (DVC) (10). Four retinal vascular plexi exist within the retina which form two complexes, the SVC and DVC (11). The CRA supplies the SVC which consists of larger arteries, capillaries, and venules. The SVC contains the nerve fibre layer plexus (NFLP) and the ganglion cell layer plexus (GCLP), with the DVC consisting of the intermediate capillary plexus (ICP) and the deep capillary plexus (DCP) (11,12). The NFLP is the most superficial plexus, which forms a dense network of capillaries exclusively in the peripapillary area, while appearing continuous with the GCLP residing in the ganglion cell layer (GCL) elsewhere (11,12). In majority of the macula the NFLP and GCLP create a continuous SVC supplying the ganglion cell complex. Near the optic nerve head the NFLP dominates the SVC and elsewhere the GCLP dominates. The NFLP also includes the radial peripapillary capillary plexus (RPCP) which supplies the dense nerve fibre layer (NFL) bundles in this region (11,12). The deeper GCLP consists of the GCL and the inner plexiform layer (IPL). In the DVC, the ICP is located in the IPL and the inner nuclear layer (INL), with the DCP found between the INL and the outer plexiform layer (OPL). The DVC is supplied via vertical anastomoses from the SVC (12).

The iBRB is a specialised endothelial barrier consisting of tight junctions between retinal vascular endothelial cells. It selectively regulates the transport of molecules across retinal capillaries (13). iBRB composition is referred to as the neurovascular unit (NVU), which is a functional collection of specialised vasculature, neurones, immune and glial cells (14). The composition of the NVU involves several neural cell types including retinal ganglion, amacrine, horizontal, bipolar and photoreceptors. Glia cells including Müller and astrocyte cells, immune cells which are microglia, perivascular cells, macrophages, vascular endothelial cells, tight junctions and pericytes (15). Cells within the NVU maintain the integrity of the iBRB.

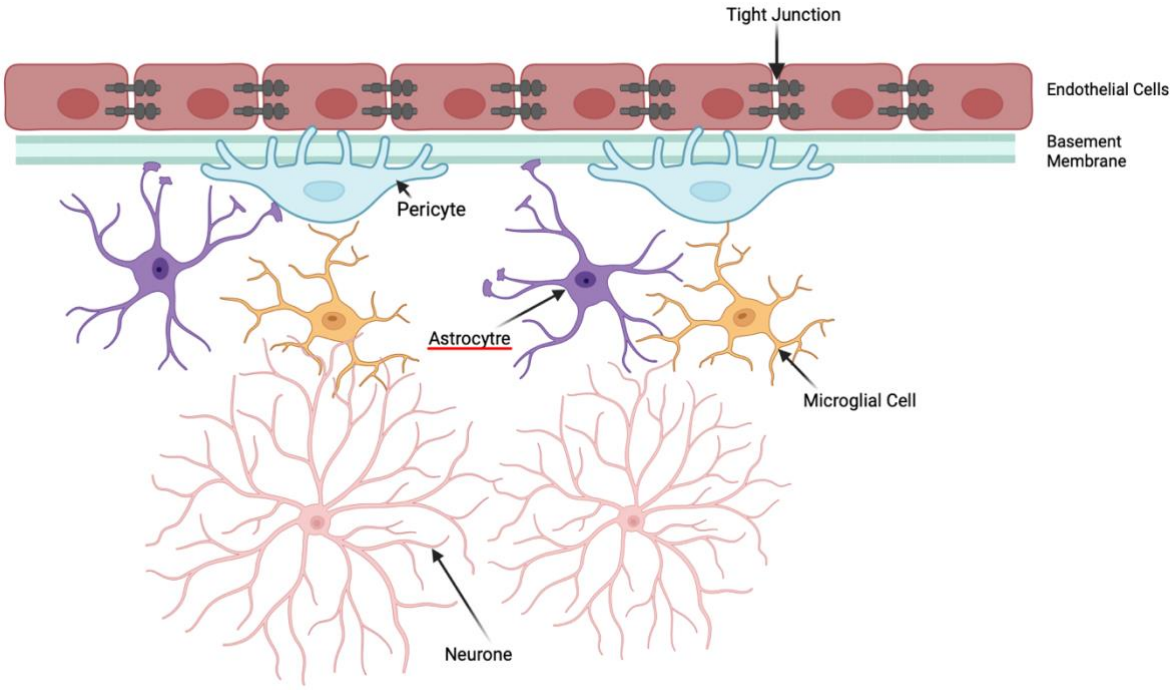

Figure 1. Schematic illustration of the retinal NVU

Illustration of the composition of the NVU and how it encompasses the iBRB. The schematic highlights the interactions between vascular endothelial cells, tight junctions, pericytes, microglial cells, astrocytes, and the basement membrane.

Chronic hyperglycaemia has a significant role in the development of DR, dyslipidaemia and hypertension have also been identified as risk factors (3,16,17). DR encompasses two stages: non-proliferative DR (NPDR) and proliferative DR (PDR). NPDR illustrates early-stage DR, it is characterised by increased vascular permeability, altered BF and capillary occlusion due to pericyte loss and disruption of tight junctions between endothelial cells (18,19). During NPDR, microaneurysms, haemorrhages and exudates are detectable by fundus photography, without any present visual symptoms (18,19). PDR signifies the advanced stage of DR, characterised by neovascularisation. Patients in this stage may suffer from extreme vision loss

due to bleeding of newly formed mutant vessels into the vitreous causing a vitreous haemorrhage, or tractional retinal detachment (18). The most prevalent loss of sight in DR patients is diabetic macular oedema (DMO), which may be present during any stage of DR (18,20). DMO is distinguished by macular swelling due to the accumulation of intraretinal fluid, caused by the breakdown of the iBRB (18,20). DMO may be present even in the absence of visual symptoms (21). Evidence from experimental DR models and in diabetic donors' retinas suggests that neurodegeneration is a hallmark factor of DR (22). Amacrine and retinal ganglion cells have been identified as the first neurones where diabetes induced apoptosis occurs, with photoreceptors also having an increased rate of apoptosis (14). Apoptosis of these cells reduces thickness of the NFL and inner retinal layers (14). Cell death within neurones in the early stages of DR activate cells within the NVU and is defined as reactive gliosis (RG). RG has been suggested as one of the primary mechanisms in the neurodegeneration process occurring in DR. Hyperglycaemia impacts astrocytes and Müller cells in the NVU, activating the functions of these glia. Specifically, activated Müller cells can produce inflammatory cytokines due to innate immune response overexpression (23). Activated Müller cells may also cause dysfunction of the iBRB through a disruption of VEGF expression (15). The breakdown of the iBRB is accelerated through the activation of glia and immune cells in the NVU which result in the secretion of pro-inflammatory cytokines and growth factors (19). These molecules include VEGF, interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8) and monocyte chemoattractant (MCP-1) (24). This dysregulation of cells within the NVU leads to enhanced vascular permeability and disruption in BF regulation. Hyperglycaemia induced breakdown of the iBRB through apoptosis and elevated production of pro-inflammatory cytokines

and growth factors, has also been strongly associated with oxidative stress (OS) (25). Abundance of reactive oxygen species (ROS) in diabetes, has been found to indirectly promote the release of pro-inflammatory cytokines and induce apoptosis through various pathways (25).

Previously it has been reported that exercise may have beneficial effects in the treatment and intervention to complications caused by diabetes in other body systems and organs. Complications within the renal system has been experienced in individuals with diabetes, which can cause diabetic nephropathy (DN). DN is a microvascular complication characterised by continuous albuminuria and a reduced glomerular filtrate rate (GFR) (26). Anatomical changes include basement membrane thickening, nodular glomerulosclerosis, mesangial expansion, and tubular atrophy (27). In a meta-analysis carried out by Cai et al, containing 18 studies and a total of 38,991 patients with DN, suggested that increased exercise levels improved the GFR and reduced the urinary albumin creatine ratio (28). Diabetes has also been observed to lead to cardiovascular complications, with a strong link associated between diabetes and cardiovascular disease (29). In a cohort study containing 1263 men with TD2, it was observed that men who exercise less frequently had a 2.1-fold increase in cardiovascular mortality compared to men who frequently exercised (30).

Exercise may have beneficial effects in other body systems. Therefore, this narrative review aims to explore the effects that exercise has on the diabetic retina, specifically surrounding neuronal apoptosis, inflammation, and OS during DR, focusing on pre-clinical animal models.

Methods

Defining the Research Question

This narrative review was conducted as an analysis trying to capture papers relevant to the effects exercise has on the diabetic retina in animal models. The patient population was animal models that diabetes had been induced in, exercise was used as an intervention in these subjects and retinal complications were assessed. Exercise type was not defined, and exercise of any type was included. Guidance and advice were sought from the medical librarian, detailing how to carry out an extensive search on various databases. The search was conducted between November 2023 and February 2024 in PUBMED and Ovid MEDLINE databases.

Defining Search Terms and Identifying Eligible Publications

Search terms were defined by the aims of the review and discussion with supervisors helped to direct the search. The following search terms were used in search 1: (((exercise) OR (physical activity)) AND (diabetic retinopathy)) AND (animal models). Results were assessed for relevance and search terms were reflected upon, dictating search terms used in the future, reflecting the scoping nature of this review. A total of 16 searches were carried out across the two databases and key words included; exercise, physical activity, DR, animal*, animal models, diabet*, retin* and vasculature. No filters, limitations or date restrictions were set on the searches. These search terms were used to capture all articles studying the effects of exercise on DR in pre-clinical, animal models. Papers were considered eligible if they were published in English and relevant to the aims of the review.

An initial screening of the titles and abstracts of potentially eligible articles determined whether they were relevant, it was necessary for a full text review

in one article to determine relevance. Relevant articles were included in the study. Listed citations of included articles were also screened for potential papers relevant to the project. Any publications that were duplicates and unrelated to diabetes, exercise, animal models or the retina were excluded. The principle was to find every relevant article whilst still having a feasible project.

The total 8 searches carried out on both PUBMED and OVID MEDLINE, and the search terms used in each search are listed below.

1) (((exercise) OR (physical activity)) AND (diabetic retinopathy)) AND (animal models) – 11 results
2) (((exercise) OR (physical activity)) AND (diabetic retinopathy)) AND ((animal*) OR (animal models)) – 36 results
3) ((((exercise) OR (physical activity)) AND (diabet*)) AND (retin*)) AND ((animal*) OR (animal model*)) – 63 results
4) ((((exercise) OR (physical activity)) AND (retin*)) AND ((animal*) OR (animal model*))) AND (retinopathy) – 120 results
5) (((((exercise) OR (physical activity)) AND (retin*)) AND ((animal*) OR (animal model*))) AND (retinopathy)) AND (diabet*) – 42 results
6) ((((exercise) OR (physical activity)) AND (diabet*)) AND ((animal*) OR (animal model*))) AND (retinopathy) – 44 results
7) (((((exercise) OR (physical activity)) AND (diabet*)) AND (retin*)) AND ((animal*) OR (animal model*))) AND (blood flow*) – 8 results
8) (((((exercise) OR (physical activity)) AND (diabet*)) AND (retin*)) AND ((animal*) OR (animal model*))) AND (vascula*) – 18 results

Results

From the database search a total of 344 results were obtained, upon reading titles and abstracts 338 articles were excluded from the review, a full text review was required for 1 article and determined it was relevant. Therefore 6 studies were included in the review. Articles were excluded from the review if they were duplicates, inaccessible, not in English and unrelated to diabetes, exercise, the retina, or animal models.

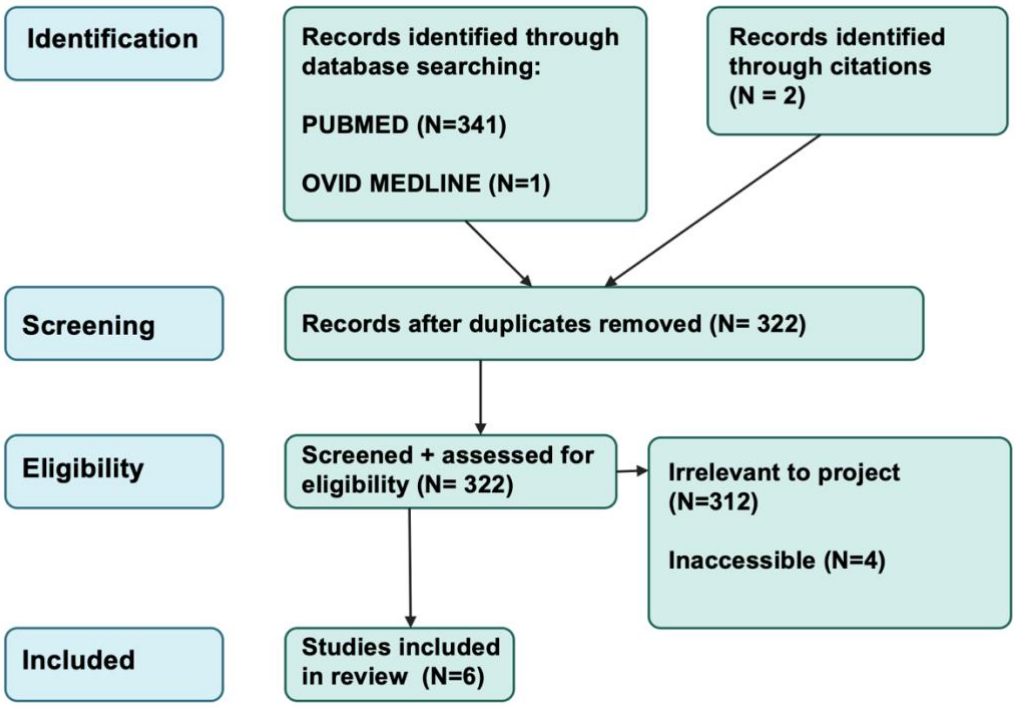

Figure 2. Prisma Flow Diagram of Search Process

PRISMA diagram indicating the study process including the identification and screening of articles for eligibility. 341 articles were identified through the PUBMED search, whilst 1 unique article was identified using Ovid MEDLINE. After the removal of duplicates 322 articles were reviewed for eligibility. 312 of these articles were excluded due to being irrelevant to the project aim, either unrelated to the retina, diabetes, animal models and or exercise. 4 articles were excluded as they were inaccessible by a pay wall. Resulting in this narrative review containing 6 articles.

Hypothesis and what was measured in each paper

Table 1. Summary of the participants, experimental protocol and duration of experiment, exercise intervention and country of origin of each article

Reference / Lead Author	Participants	Experimental Protocol / duration	Intervention	Country of origin
(31) Kim et al, 2013	32 Male Sprague-Dawley rats	Streptozotocin (STZ) induced diabetes – 6-week protocol	Treadmill exercise	Korea
(32) Allen et al, 2018	120 Long Evans rats	STZ induced diabetes – 8-week protocol	Treadmill exercise	USA
(33) Ji et al, 2013	30 Male Sprague-Dawley rats	STZ induced diabetes – 1 week protocol	Treadmill exercise	Korea
(34) Dantis Pereira de Campos et al, 2020	35 Swiss mice	High fat diet (HFD) induced obesity and hyperglycaemia – 1-week protocol	Combined exercise protocol – strength exercise (weighted climb) and treadmill running	Brazil
(35) Kim et al, 2015	104 Male C57BL/6J mice	Mice aged 22 months – 12-week protocol	Treadmill exercise	Korea
(36) Sadeghian et al, 2021	48 Female Wistar rats	Ad libitum HFD and a low dose STZ injection – 8-week protocol	Swimming exercise	Iran

Results of papers

Table 2. Summary of the outcomes from each article, including what was measured, how was it measured and did exercise result in a significant change of the molecule.

Theme	What was measured	How was it measured	What was the result from exercise (↑, ↓, -)
Apoptosis	Blood glucose level TUNEL cells	Blood glucose test TUNEL staining	Blood glucose –

	Caspase-3-positive cells Bax Bcl-2 p-Akt Akt	Immunohistochemistry Western blot analysis	TUNEL cells ↓ (P<0.05) Caspase-3 ↓ (P<0.05) Bax ↓ (P<0.05) Bcl-2 ↑ (P<0.05) p-Akt ↑ (P<0.05) Akt – p-Akt/Akt ↑ (P<0.05)
TrkB signalling pathway	Blood glucose Visual Function Retinal Function	Freestyle handheld blood glucose meter Virtual optokinetic system Electroretinogram	Blood glucose – Visual acuity↑ ($p < 0.001$) Spatial frequency ↑ ($p < 0.001$) Contrast sensitivity↑ ($p = 0.003$) Oscillatory potential waveforms ↑ ($p < 0.001$) ERG flicker amplitudes ↑ ($p < 0.001$) ERG implicit times ↑ ($p < 0.001$)
Apoptosis and VEGF	Blood Glucose Caspase-3 positive cells TUNEL cells VEGF expression	Blood glucose tester Caspase-3 Immunohistochemistry TUNEL staining Western blot	Blood glucose - Caspase-3 positive cells ↓ ($p < 0.05$) TUNEL cells ↓ ($p < 0.05$)

			VEGF expression ↓ ($p < 0.05$)
Pro and anti-inflammatory profile	Physiological and metabolic parameters Inflammatory serum profile Hepatic insulin sensitivity Proinflammatory cytokines	Insulin tolerance test Glucose tolerance test Immunoblotting assay Insulin assay	Fasting hyperglycaemia ↓ ($p < 0.05$) Insulin tolerance test ↓ ($p < 0.05$) IL-1β ↓ ($p < 0.05$) TNF-α ↓ ($p < 0.05$) IL-6 - IL-10 ↑ ($p < 0.05$) pTAK1 -
Oxidative stress	Carboxymethyllysine (CML) 8-hydroxy-2'-deoxyguanosine (8-OHdG) Nitrotyrosine	Immunohistochemical staining for OS markers	CML ↓ ($p < 0.05$) 8-OHdG ↓ ($p < 0.05$) Nitrotyrosine ↓ ($p < 0.05$)
Inflammation, oxidative stress, VEGF expression	Glucose homeostasis parameters miR-132 miR-146a Inflammatory factors Oxidative stress biomarkers MMP-2 VEGF NF-κB p-ERK	Polymerase chain reaction techniques Western blotting Lipid peroxidation assay Assessment of GSH – Ellman method Enzyme-linked immunosorbent assay	Fasting blood glucose ↓ ($p < 0.05$) miR-132 ↓ ($p < 0.01$) miR-146a ↓ ($p < 0.01$) IL-1β ↓ ($p < 0.05$) TNF-α ↓ ($p < 0.05$) Malondialdehyde ↓ ($p < 0.01$) Glutathione (GSH) ↑ ($p < 0.01$) MMP-2 ↓ ($p < 0.05$)

			VEGF ↓ ($p < 0.05$) NF-κB ↓ ($p < 0.05$) p-ERK ↓ ($p < 0.01$)
Key ↑ Statistically significant increase ↓ Statistically significant decrease - No significant change			

Kim et al, 2013, investigated the effects that treadmill exercise had on apoptotic cell death and protein kinase B (Akt) in diabetic rat retinas. Rats were split into four groups; control, control + exercise, streptozotocin (STZ)-induced diabetes and STZ-induced diabetes + exercise. An intraperitoneal injection of STZ induced diabetes, by inducing pancreas β-cell toxicity resulting in the destruction of β-cell islets. It is commonly used to induce diabetes in animal models (37). The treadmill exercise group ran on treadmills for 30 minutes a day, 5 days a week over a six-week period. The rats ran at a speed of 3 m/min for 5 minutes, 5m/min for the next 5 minutes and 8m/min for the remaining 20 minutes. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) cell staining, and caspase-3 immunohistochemistry were performed on the retinas. TUNEL cell staining detects fragmented DNA, as a measure of cell death by apoptosis (38). Caspase-3 immunohistochemistry identifies caspase-3 positive cells. Caspases are cysteine proteases regulating apoptosis in a number of cells including neurones and are sensitive to OS (39,40). Caspase-3 is an executioner caspase, functioning downstream as a death signal mediating the mechanisms of apoptosis through the activation of other caspases (41). STZ induced diabetes enhanced the expression of TUNEL and caspase-3 cells, with treadmill exercise suppressing both. Western blot analysis was performed to detect the expression of Bax, Bcl-2 and Akt expression. The Bcl-2 protein family can regulate apoptosis, as members of this

family consist of anti-apoptotic and pro-apoptotic molecules (42). Bcl-2 is a molecule that promotes apoptosis whereas Bax is a molecule which inhibits apoptosis (43). STZ induced diabetes increased the expression of Bax and treadmill exercise significantly reduced Bax expression. Bcl-2 expression was increased in diabetic retinas; however, treadmill exercise significantly increased the expression of Bcl-2. P-Akt/Akt pathway contributes to neuronal survival by suppressing apoptosis, preventing cytochrome c release and apoptosis inducing factors from mitochondria (44). STZ induced diabetes reduced p-Akt expression, whilst treadmill exercise significantly increased p-Akt expression. No significant change occurred in Akt expression between the STZ induced diabetes group and diabetic exercise group. These changes significantly increased the p-Akt/Akt ratio within diabetic retinas of the treadmill exercise group, increasing the expression of anti-apoptotic factor p-Akt.

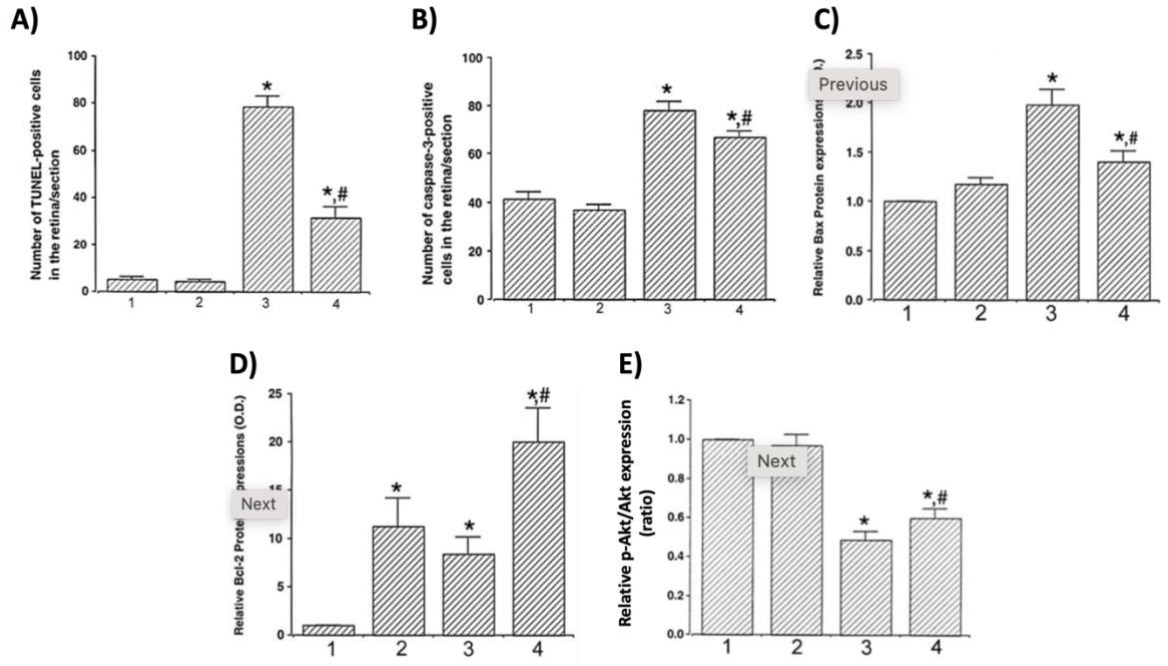

Figure 3. Kim et al, 2013, effects of treadmill exercise on the expression of different apoptotic biomarkers and Akt within the retina.

A) Quantification of TUNEL-positive cells in the retina. **B)** Quantification of retinal caspase-3-positive cells. **C)** Bax protein expression in the retina. **D)** Bcl-2 protein expression in the retina. **E)** p-Akt / Akt ratio in the retina. (1) control, (2) control and exercise, (3) STZ-induced diabetes, (4) STZ-induced diabetes and exercise. *p < 0.05, vs control; #p < 0.05, vs STZ-induced diabetes, determined by one-way ANOVA. Data is presented as the mean ± SEM.

Allen et al, 2018, exqamined the effects treadmill exercise had in the treatment of STZ induced diabetes in male Long Evans rats, specifically how the Tropomyosin receptor kinase B (TrkB) pathway promotes protective effects in the retina. TrkB signalling pathway promotes neuronal survival, growth, and differentiation through the activation of downstream signalling cascades (45). An intravenous injection of STZ induced diabetes. Diabetic rats were distributed into four groups; inactive + vehicle, active + vehicle, inactive + ANA-12, and active + ANA-12. Non-diabetic rats used as controls were allocated to these

same four groups. The treadmill exercise groups ran five times per week for 30 minutes, at a pace of 15m/min for an 8-week period. ANA-12, a direct and selective TrkB receptor antagonist or vehicle was injected 2.5 hours before exercise, to determine the effects the TrkB signalling pathway had on the protective mechanisms (46). Spatial frequency and contrast sensitivity were measured using a virtual optokinetic system. After 8 weeks, diabetic and inactive rats had a significant decline in spatial frequency and contrast sensitivity opposed to non-diabetic controls. The treadmill exercise groups had preserved spatial frequency thresholds and contrast sensitivity higher than diabetic, inactive rats. ANA-12 injections in the exercise group suppressed the effects of exercise and values were indistinguishable from the inactive diabetic groups. An electroretinogram (ERG) was used to assess retinal function, oscillatory potential (OP) waveforms experienced delays in diabetic inactive rats, which was not observed in diabetic and exercised rats. ANA-12 injections in the exercise group abolished the protective effect exercise had. ERG flicker amplitudes and implicit times at 8 weeks also experienced significant delays in inactive diabetic rats. The diabetic exercise group had maintained flicker amplitudes and implicit times, which were almost identical from non-diabetic controls.

Ji et al, 2013, investigated effects of treadmill exercise on apoptotic cell death and VEGF expression in STZ induced diabetic rats' retinas. Rats were allocated to three groups; control, STZ induced diabetes and STZ induced diabetes and treadmill exercise. Exercised rats ran on a treadmill, 30 minutes per day, for one week. The running speed was 2 m/min for 5 minutes, 5 m/min for 5 minutes and 8 m/min for the remaining 20 minutes. Western blotting was used to measure VEGF levels, a potent angiogenic growth factor involved in retinal

neovascularisation (47). Results indicate that diabetes increased the levels of VEGF and treadmill exercise significantly reduced levels of VEGF in the retinas. TUNEL staining identified that diabetes increased cell death by apoptosis in the retinas whilst treadmill exercise significantly reduced apoptosis in the retinas of diabetic rats. Immunohistochemistry was used to visualise retinal caspase-3 positive cells. Diabetes increased retinal caspase-3 expression and treadmill exercise significantly reduced the expression of caspase-3 in diabetic retinas.

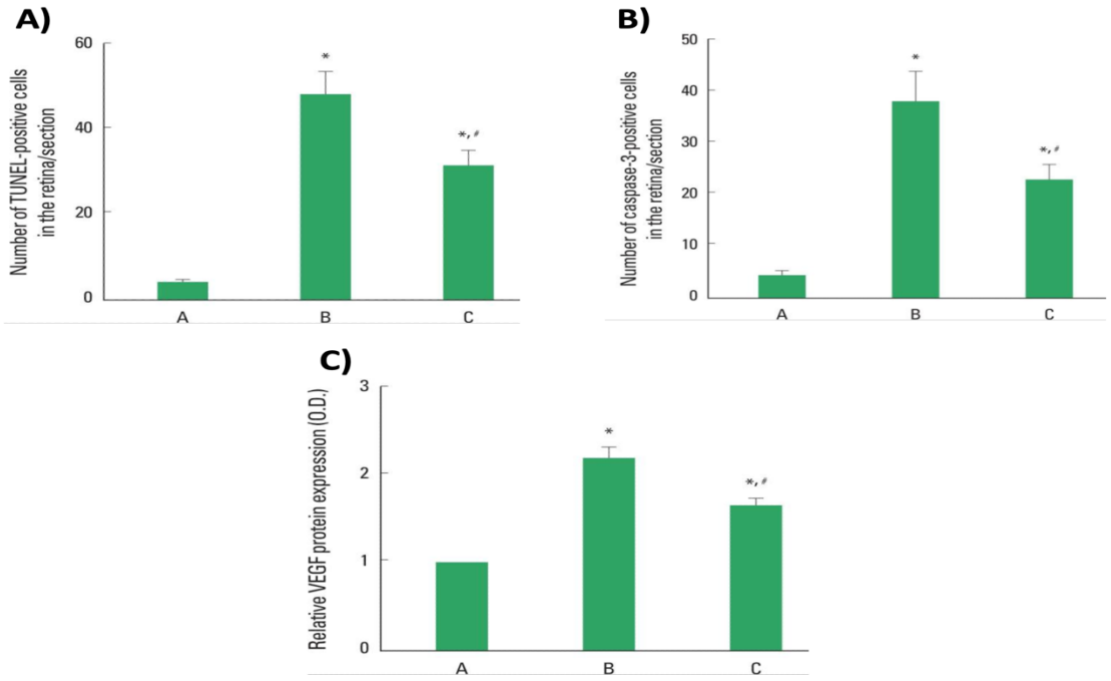

Figure 4. Ji et al, 2013, effects of treadmill exercise on the expression of apoptotic biomarkers and VEGF expression.

A) Effects of treadmill exercise on the expression of TUNEL–positive cells in the retina.

B) Effects of treadmill exercise on caspase-3-positive cell expression within the retina.

C) Effects of treadmill exercise on VEGF expression within the retina. (A) Control group, (B) STZ-induced diabetes group, (C) STZ-induced diabetes group and treadmill exercise. *p < 0.05, vs control group; #p < 0.05, vs STZ-induced diabetes group, determined by one-way ANOVA. Data expressed as the mean ± SEM.

Dantis Pereira de Campos et al, 2020, investigated combined short term exercised exercise effects on the pro and anti-inflammatory profile in retinas of obese mice. Swiss mice were divided into 3 groups; control, sedentary obese (SOB) and trained obese (TOB). Mice consumed a HFD for 10 weeks to induce obesity and hyperglycaemia, and randomly assorted into SOB and TOB groups. The combined exercise protocol for TOB group consisted of a climbing series and a climb with 70% of their maximum voluntary carrying capacity. Mice rested 90 seconds before they ran for 30 minutes on a treadmill at 75% of their exhaust velocity. The exercise protocol lasted 7 days. An immunoblotting assay was used to evaluate the pro and anti-inflammatory cytokines, IL-1β, tumor necrosis factor-alpha TNF-α, IL-6 and interleukin-10 (IL-10), levels in the retina (48). SOB had increased levels of the pro-inflammatory cytokines IL-1β, TNF-α, IL-6, and exercise was found to reduce these parameters. Exercise training was also found to significantly increase levels of IL-10, a pro-inflammatory cytokine, no significant increases occurred in SOB occurred.

Kim et al, 2015, investigated the effects treadmill exercise had on OS in the retina. Male C57BL/6J were aged for 22 months and separated into two groups old control and old exercise group. Control mice did not exercise, mice in the exercise group ran on a treadmill 3 times a week for 12 weeks. For the first few week's mice ran at a pace of 5 metres per minute, which gradually built to 12 metres per minute by week 12. Immunohistochemical staining was used to identify OS markers, CML, 8-OHdG and nitrotyrosine. Local OS and damage to tissues through protein oxidation can be generally marked by CML (49). 8-OHdG is formed from the oxidation of guanine and is a indicator of oxidative DNA damage (50). Nitrotyrosine formation is an indicator of nitro-OS and can be detected when nitric oxide and oxidants are formed (51). Levels of all OS

markers CML, 8-OHdG, and nitrotyrosine, were increased in aged mice suggesting oxidative damage in the retina. The treadmill exercise group experienced significant decreases in levels of all 3 markers which suggests exercise may inhibit oxidative damage.

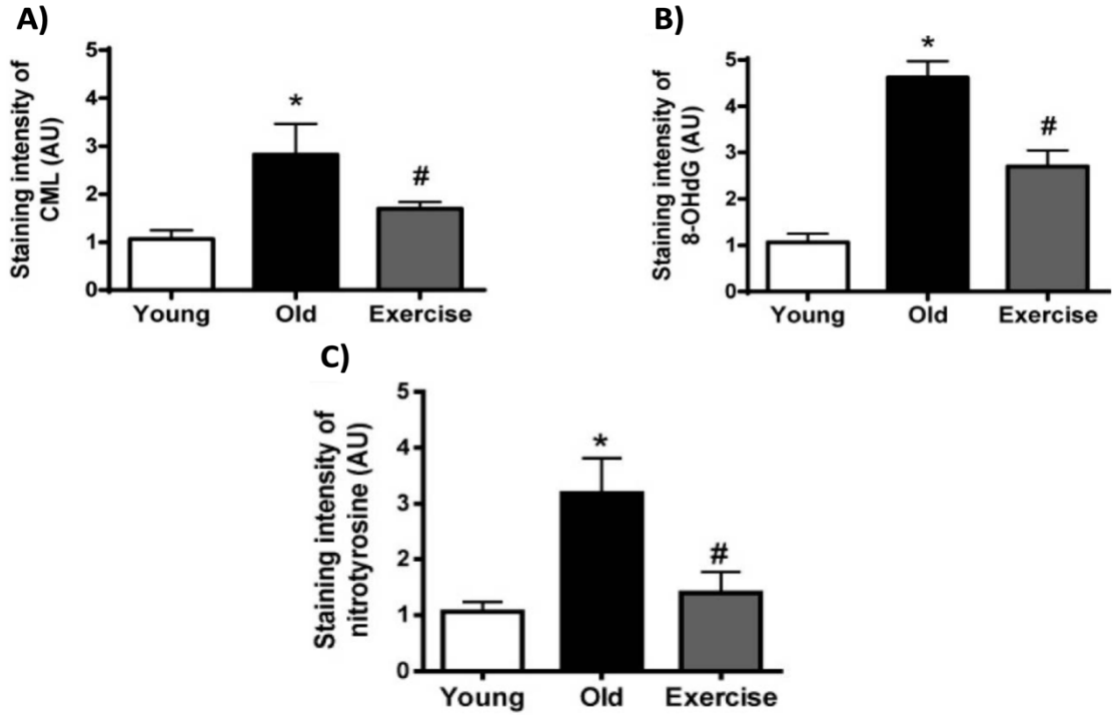

Figure 5. Kim et al, 2015, effects of treadmill exercise on oxidative stress markers CML, 8-OHdG and nitrotyrosine.

A) Effects of treadmill exercise on CML formation within the retina. **B)** Effects of treadmill exercise on the formation of 8-OHdG within the retina. **C)** Effects of treadmill exercise on the formation of retinal nitrotyrosine. Young control, old, and old exercised groups are represented in the graphs. *p < 0.05, vs the young control group; #p < 0.05, vs the old group, determined by one-way ANOVA. Data is expressed as the mean ± SEM.

Sadeghian et al, 2021, investigated the effects of swimming training on neovascularisation factors within the retina. Wistar rats were split into 6 groups of 8 rats, however the only relevant groups needed for this review are: sham group, ovariectomised group (OVX.D) with diabetes and ovariectomised group

with diabetes and exercise (OVX.D.E). Diabetes was induced by an ad libitum HFD for four weeks and an injection of STZ. Rats in the exercise group underwent 8 weeks of swimming training, rats swam 6 days a week for 60 minutes for the 8-week programme. PCR techniques were used to measure the expression of miR-132 and miR-146a, micro RNAs involved in the upregulation of neovascularisation and inflammatory cytokines in the retina (52,53). Increased levels of both were recorded in OVX.D group with swimming training significantly reducing the levels of both. Western blotting assessed VEGF and NF-κB expression in the retina. The diabetic group had significantly increased levels of both VEGF and NF-κB, with swimming training significantly decreasing these levels. An enzyme-linked immunosorbent assay determined IL-1β and TNF-α levels in the retina, the diabetic group caused a significant increase on both IL-1β and TNF-α, whilst swimming exercise significantly reduced these levels. Oxidative stress markers were also measured through the levels of MDA and GSH. Significant increases in both MDA and GSH occurred in the diabetic groups, with swimming training significantly reducing these levels.

Discussion

Papers included in the review suggested three main themes that exercise had beneficial effects on: OS, apoptosis, and inflammation. These themes are associated with the pathophysiological mechanisms of DR.

Oxidative Stress

Due to the large quantities of polyunsaturated fatty acids, high oxygen uptake, and continuous exposure to ROS producing UV and visible light, the retina is vulnerable to OS (54). OS has a critical role in DR pathophysiology, as excessive ROS accumulation due to hyperglycaemia impairs retinal cells and vessels through apoptosis, inflammation, and growth factors (55). Excessive ROS Despite Kim et al, 2015, using models of aged mice, it suggests the beneficial effects of exercise in the retina which may be transferable in hyperglycaemic induced OS. Studies suggested that treadmill and swimming exercise causes significant reductions in OS markers in the retina and potential reductions in OS related damage. Regular levels of exercise have been found to promote the increased production of antioxidant enzymes and GSH levels in aging skeletal muscles, which may limit free radical production and therefore OS related damage (56). Raza et al, 2016, demonstrated that exercise induces beneficial effects on pancreatic OS in type 2 diabetic rats through improvements in the antioxidant, GSH, levels and mitochondrial function (57). Annie et al, 2022, demonstrated that there were significant increases of OS induced proteins within the liver and kidney of GK diabetic rats. These levels were reduced to control rats' levels after exercise treatment (58). Antioxidant indicator proteins were significantly reduced in the liver and kidneys of these rats and exercise significantly recovered the levels of these proteins (58). These results suggest

the beneficial effect that exercise has in the prevention of OS and may potentially be through the increase of antioxidant levels.
Although exercise has been suggested to be beneficial in the reduction of OS, overtraining and rigorous exercise has been suggested to be harmful. Overtraining may lead to the reduction of antioxidant enzymes and GSH and may induce OS related damage through the production of free radicals (59).

Apoptosis

Apoptosis is a recognised characteristic underlying the pathophysiological mechanisms of DR (60). Hyperglycemia induces retinal pericyte and endothelial cell death through apoptotic pathways (61,62). This review highlights the effects of hyperglycemia on the increased expression of TUNEL cells, caspase-3 cells, and the protein Bax. Previous studies also suggest the expression of the pro-apoptotic molecules, caspase-3 and Bax, were increased in diabetes (63,64). TUNEL staining has also proven useful identifying apoptosis in amacrine and retinal ganglion cell, with increased levels of TUNEL cells detected in diabetic rats (65). High glucose levels induce OS, which leads to an increase of caspase-3 activity (55). ROS accumulation in mitochondria due to hyperglycemia, increases mitochondrial pore permeability triggering cytochrome c to release from retinal mitochondria inducing apoptosis through a caspase cascade (66,67). High glucose levels also increase Bax expression and permit the translocation of Bax from the cytosol to the mitochondrial membrane, further inducing cytochrome c release from the retinal mitochondria (68).
Cytochrome c initiates apoptosis through activating caspase-9, which further activates caspase-9 downstream through a biological cascade leading to the fragmentation of DNA (69). Treadmill exercise in this study was found to

significantly reduce the expression of TUNEL, caspase-3 cells and Bax expression in the retinas of STZ induced diabetic rats. A study carried out on rats by Zhang et al, recorded that swimming exercise for 8 weeks provoked a significant reduction in TUNEL and caspase-3 expression in the heart after ischemia or a reperfusion injury (70). Treadmill exercise was also found to significantly suppress both Bax and caspase-3 expression in the hippocampus of rats after suffering traumatic brain injury (71). These observations suggest the protective effects that treadmill exercise may have in preventing retinal apoptotic death through the reduction of pro-apoptotic factors.

Treadmill exercise in the present study significantly enhanced the expression of Bcl-2 and p-Akt / Akt ratio. Bcl-2 has been reported to prevent the release of cytochrome c from mitochondria during apoptosis (68,72,73). Cycling exercise on rats was reported to increase the expression of Bcl-2 in the spinal cord and reducing caspase-9 expression (74). Treadmill exercise increased Bcl-2 expression in the hippocampus of rats after traumatic brain injury (71). Akt activation by Phosphoinositide 3-kinase (PI3K) has been found to inhibit apoptosis through reducing the expression of caspase-3 and -9, promoting cell survival (75,76). P-Akt phosphorylates the apoptotic factor Bad, preventing the translocation of Bax to the mitochondrial membrane, which will inhibit apoptosis (75). Um et al revealed that treadmill exercise enhanced levels of p-Akt reducing neuronal apoptosis in transgenic mice models of Alzheimer's disease (77).

Pro and anti-inflammatory Profile and VEGF

Chronic inflammation coupled with growth factors including VEGF have a crucial role in the BRB breakdown and angiogenesis in the development of DR (78). The release of inflammatory mediators is also related to the progression

of apoptosis in the pathogenesis of DR (79). Hyperglycemia induced OS causes the accumulation of ROS mediating the proliferation and migration of microglia cells from the inner to outer retina. Microglia cells will produce and secrete pro-inflammatory factors including IL-1β, TNF-α, NF-κB, IL-6 and MMP-2 (55,78,80). Inflammatory cell accumulation may lead to the destruction of retinal tissues and capillaries, leading to the increased vascular permeability in the retina during DR (81). This review illustrates that treadmill exercise suppressed the expression of pro-inflammatory factors IL-1β, TNF-α, NF-κB, IL-6 and MMP-2, whilst increasing the expression of the anti-inflammatory factor IL-10. Studies on overweight and obese adults concluded that aerobic exercise training reduced of IL-6 and TNF- α expression, whilst resistance training upregulated IL-10 levels (82). A review study also found that combined exercise for 8 weeks significantly decreased IL-1β and TNF-α levels in sedentary and obese adults (83). The decrease of IL-1β and TNF- α through exercise may have suppressive effects on the expression of Matrix-metalloproteinases, particularly MMP-2. MMP-2 damages retinal mitochondria, increasing apoptosis in retinal cells and assist in angiogenesis (84,85).

Hypoxic conditions caused by the loss of retinal capillaries and increased retinal permeability mediates the production of VEGF from retinal cells, an angiogenic factor in DR (81,86). VEGF is the principal growth factor accelerating retinal endothelial cell growth and vascular permeability, with significant VEGF expression in diabetic rat retinas (87,88). Erekat et al, 2014, demonstrated treadmill exercise improved VEGF expression in the cardiac muscles of STZ-induced diabetic rats (89).

As suggested the beneficial effects of exercise by reducing markers of OS, apoptosis, and inflammation within the retina. In the papers, exercise did not

have a significant effect on the regulation of hyperglycaemia, suggesting beneficial effects were not through blood glucose regulation. Mechanisms of exercises beneficial effects remain unknown and will require further research.

Strengths and Limitations

The study included 6 papers, with the same recurring themes appearing. Apoptosis, OS, inflammatory profile, and VEGF were common themes amongst the 6 papers. Kim et al, 2013 and Ji et al, 2013, demonstrated that treadmill exercise significantly reduces apoptotic factors within the retina. Dantis Pereira de Campos et al, 2020, Sadeghian et al, 2021, and Ji et al, 2013, suggested that treadmill and swimming exercise significantly reduced the pro-inflammatory profile and VEGF expression within diabetic retinas of animal models. Sadeghian et al, 2021 and Kim et al, 2015, demonstrated treadmill and swimming exercise significantly reduced OS markers in the retina. Multiple papers suggesting the same effects that exercise has a positive effect on DR disease mechanisms is positive and suggests exercise has a therapeutic effect on DR.

Despite showing promising results from the studies, all the studies included in the review are short term, with the longest being 12 weeks. Longer term effects that exercise has on the retina are not able to be concluded from these papers requiring the need for longer term studies. Inaccuracies in some of the papers regarding apoptosis were also present, the numbers of TUNEL cells reported in Kim et al, 2013 and Ji et al, 2013 studies were abnormally large for such time scale they were recorded in. Therefore, inaccuracies may also be present in the exercise group. Two studies, Dantis Pereira de Campos et al, 2020 and Kim et al, 2015, carried out experiments on obese mice and naturally aged mice. Although underlying mechanisms of OS, inflammatory factors and

growth factors effected by exercise in these groups is similar to diabetic model retinas, by not using diabetic model animals the results may have inaccuracies. These papers were kept in the review as they demonstrated retinal changes of OS, inflammation, and growth factors during exercise. The sample sizes of animals included in each study were also small, and may not be reflective of population statistics, effecting reliability of results.

Conclusion

As DR incidence rates increases, DR continues to be a prevalent vision threatening complication of DM. This review captured relevant pre-clinical studies on the effects that exercise had on the diabetic retinas of animal models. Paper results in this review indicated a potential therapeutic effect that exercise may have on the pathophysiological mechanisms of DR; apoptosis, inflammation, VEGF, and OS. Results included short term papers, which can be used as preliminary guidelines to get funding for larger, longer-term studies before clinical studies are possible. The inclusion of animal model studies in this review was determined to allow for better understanding of the disease mechanisms of DR instead of population level studies. In future studies, larger time frames and sample sizes are necessary to gather long term effects of exercise, and to be reflective of the population. Before moving to clinical studies, it is necessary for a long-term study, carried out on a large animal population with various exercise training protocols. To help determine most the most effective exercise and duration of exercise which bests targets DR disease mechanisms. These studies will help to determine new targets for treatment of DR disease mechanisms. Potential identified therapeutic targets may be the basis for clinical studies into the effects of exercise training on DR in humans, to help prevent DR in the future.

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