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REVIEW ARTICLE

Insulin Impact in Glaucoma Neurodegeneration and Vascular Dysfunction

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[1] Laboratory neurogenesis research and development

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ABSTRACT

Neurodegeneration in glaucoma remains a significant challenge, even with efforts to lower intraocular pressure (IOP). Many patients continue to experience visual field loss. This review will highlight the role that insulin signalling plays in neurodegeneration and also in vascular dysfunction.

Neurodegeneration glaucoma and Alzheimer's disease (AD) share many identical characteristics, deposits of beta-amyloid, cell apoptosis, Tau hyperphosphorylation, NFT formation, and mitochondrial dysfunction with elevated OS.

Vascular autoregulation in glaucoma is abnormal with a high level of endothelin-1 (ET-1) and impaired nitric oxide (NO) signaling.

There is insulin resistance by an increase of serine of insulin receptor -1 phosphorylation (p(ser)IRS-1) instead of tyrosine –IRS-1 phosphorylation (p (Tyr) IRS-1) causing alteration in insulin signaling.

C-peptide presence in the brain and insulin detection in neuron culture reinforce the evidence of insulin central secretion.

In this review, we will see the capacity of insulin intravitreal injection to promote visual function, restore the balance between NO/ET-1 secretions, and ameliorate neurite outgrowth and function.

1-Introduction

Glaucoma is a progressive ocular neurodegeneration disease with vascular dysfunction and first leads to blindness. More than 76 million cases in 2020 were observed and could reach 111.8 million in 2040. [1, 2] It has a prevalence of approximately 2% in patients over 40 years and 10% in patients over 80 years. [3]

Ocular hypertonia (OHT) represents the major risk factor, as a consequence of trabecular meshwork damage and resistance, leading to an unbalance between production and evacuation of aqueous humor, resulting in a gradual retinal ganglion cell loss generated by apoptosis, which is the precursor of neurodegeneration process, and a whole damage in optical nerve.

There is a proof with OCTA (angio OCT) that ocular blood flow is abnormal with vessel narrowing OCTA shows a reduction in blood flow particularly in the optic nerve head and macula.

Vascular dysregulation contributes to the progression of the disease there is a delicate balance between nitric oxide and endothelin concentration which are under the control of the insulin signaling pathway.

Insulin is a key factor in neuroprotection, in this review we will see the insulin impact in neurite outgrowth and mitochondrial biogenesis.

Glaucoma is represented as a socio-economic health problem, getting worse over time by aging. With more than 50% of patients, do not know that they are affected by this neurodegenerative pathology.

2-Vascular dysfunction in Neurodegeneration disease (glaucoma and Alzheimer's disease (AD)

Glaucoma shares similar pathophysiological mechanisms as other neurodegenerative diseases, particularly with AD. **(4)** Glaucoma is a progressive optic neuropathy resulting from decreased axonal transportation with subsequent retinal ganglion cells (RGC) loss followed by irreversible visual loss. [5]

There is evidence to support the role of vascular dysfunction in neurodegeneration. Functional magnetic resonance imaging (MRI) shows alteration in the oxygen blood of the visual cortex. Intracranial vascular changes were also observed in glaucoma patients. Cohort studies using MRI have shown increased white matter hypointense lesions in primary open-angle glaucoma (POAG) patients. [6]

In Normal-tension glaucoma (NTG) there are different central small vessel ischemic changes, the potential ischemic pathophysiological basis in law-tension glaucoma is further supported by the finding of a greater extent of cerebral infracts. **(7)** Glaucoma patients were noted to have lower middle cerebral artery blood flow velocities and absence of vaso-reactivity to hyperoxia compared with control. This funding suggested that diminished central visual function may be the first manifestation of white-spread cerebrovascular insufficiency. [8] The high metabolic nature of the retina necessitates a continual supply of metabolic and removal of oxidative waste vessel [9] caliber measurement indicating that arterial vessel narrowing is associated with optic nerve damage and severity of optic neuropathy. [10] Optical Coherence Tomography (OCT) angiography in glaucoma patients shows reduced vessel density in retinal capillary layers. Glaucoma is often associated with an unmet need for metabolites and O2 due to insufficient blood flow. [11]

A- VASCULAR REGULATION:

The blood flow autoregulation system allows a supply of metabolites despite fluctuations in ocular perfusion pressure. [12] However, autoregulation is achieved by changes in the tone of blood vessels; the arterials contract, and relax in response to an increase, or decrease in intravascular pressure [13,14]. Retinal vessels have regulatory mechanisms that allow blood flow to meet the metabolic demands of nerve cells [15]. Moreover, there is a delicate balance between nitric oxide and endothelin (ET) concentrations.

B- IMPAIRED REGULATION OF BLOOD FLOW IN GLAUCOMA:

Vascular autoregulation in glaucoma patients is abnormal. [14] Glaucoma patients have a high level of ET in aqueous humor. Intravitreal injection of endothelin-1 (ET1) leads to a loss of RGC and increases the excavation of the optic disc through a loss of the Retinal Nerve Fiber Layer (RNFL). [16]

Blocking ETA and ET_B receptors increases blood flow in the retina and choroids. [17] In a prospective study, a significant dilatation of retinal vessels was shown in after 8 days of administration of 500mg intravenous bosentan (an antagonist which bloc ET_A and ET_B receptors). Retinal blood flow and viscosity increased up to +45% after administration of bosentan. [18] Impaired nitric oxide (NO) signaling has been found in aqueous humor in patients with POAG as well as reduced Cyclic guanosine monophosphate (c- GMP) a molecule that signals the production of NO. [19]

C- NEUROVASCULAR ACTIVITY AND ITS IMPACT ON NEURODEGENERATION:

The increase in neuronal activity enhances energy demand and boost blood flow in this area. Neuronal activity and blood flow are tightly coupled in the central nervous system in a phenomenon known as functional hyperemia. [20]

Neuronal activity leads to neuronal signaling to the blood vessels of neighboring astrocytes, leading to vasoactive agents release thus increasing blood supply. [21] Generally, a peak in neuronal activity leads to an increase in intracellular Ca^{2+} which generates NO.

NO diffuses to the endothelial cells of local blood vessels, activating K^+ channels which leads to vessel dilation and increased blood flow. Dysfunctional NO signaling is involved in glaucoma neurodegeneration and endothelial dysfunction. NO inhibitors reduce vasodilation in the retina and NOH. [22] Pericytes express several types of muscle contractile proteins [23] and are responsive to the vasoactive molecule.

An increase in neuronal activity causes synaptic release of glutamate activating N -methyl-D-aspartic acid (NMDA) receptors on neurons leading to intracellular NO rise.

NO can activate big calcium channel ($BKCa^{2+}$) channels directly. [24] Or indirectly through NO-derived cGMP.Ca^{2+} influx leads to vasorelaxation (vasodilatation) and increased blood flow. The neurovascular unit is represented by neurons (RGC), microglia astrocytes, pericytes, and endothelial cells. **(Figure 01)**

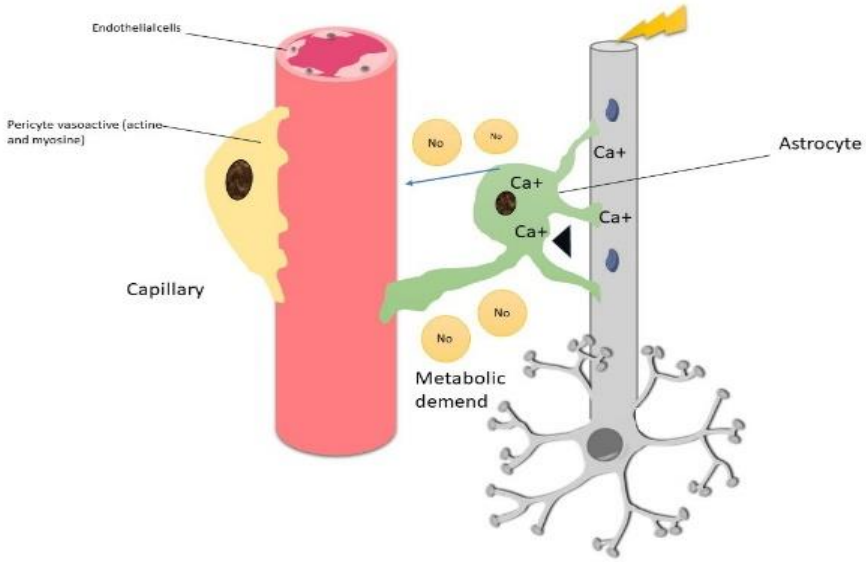


Figure 01:. The figure presents the neurovascular unit capillary, astrocytes, pericyte, and neurones, Neuronal activity leads to an increase of intracellular Ca^{2+} to generate NO

D- INSULIN SIGNALING PATHWAY IN VASCULAR REGULATION:

The biological actions of insulin are initiated by binding to surface receptors of tyrosine kinase of insulin. The activated insulin receptors phosphorylate intracellular substrates IRS-1 and shc, which serve as docking proteins for downstream signaling molecules. [25]

- Insulin pathway stimulated production of NO (PI3K):

Insulin receptor phosphorylation of IRS1 which then binds and activates PI3Kinase leading to phosphorylates and activation of PDK-1 which in turn phosphorylates and activates AKT. AKT phosphorylates eNOS at Ser resulting in increased eNOS activity and NO. [25]

- The insulin pathway to stimulate secretion of ET-1 (MAPK):

Activation of the IR by insulin favors the binding of shc to src homology2 domain of growth factor receptor-bound protein (GRB2) this is to activation of the pre-associated guanine nucleotide exchange factor son of the endless (SOS). The activated SOS promotes the removal of GDP from Ras, which then initiates a kinase phosphorylation cascade involving Raf, thus activate MAPK. [26] The MAPK pathway is responsible for biological actions related to growth, mitogenesis, and differentiation and in secretion of ET-1. **(Figure02)**

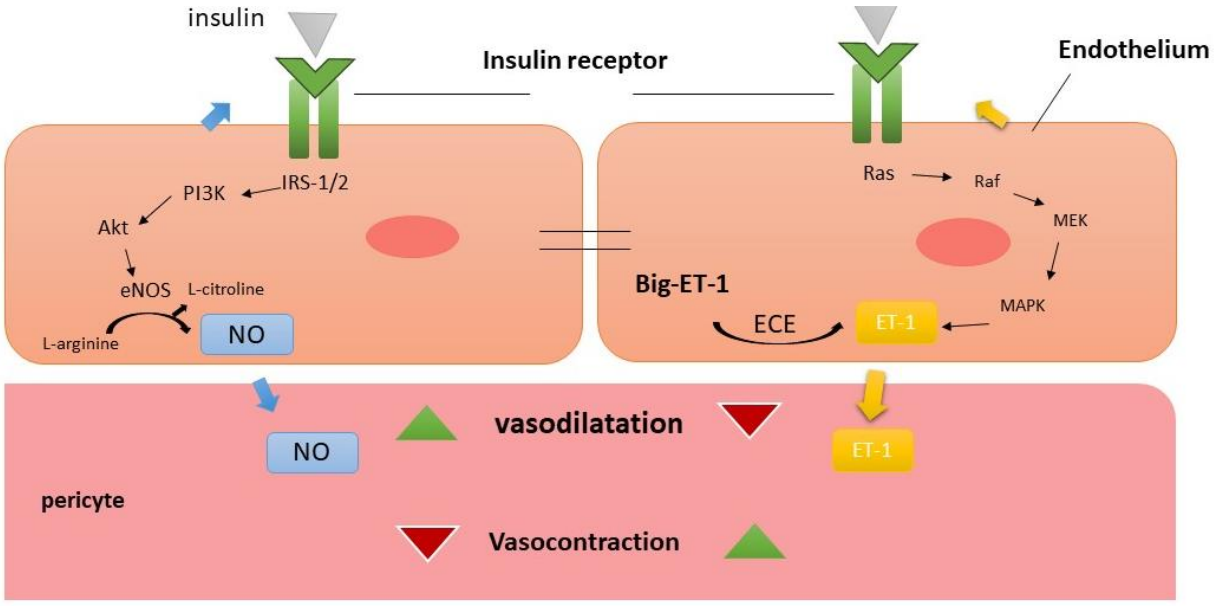


Figure 02: showing Insulin signalling pathway to produce both of nitric oxide (NO) and secretion of endothelin-1 (ET-1). NO reduces expression of adhesion molecules in endothelium, promotes vasorelaxation, ET-1 increases the expression of adhesion molecules, vasoconstriction, migration, and proliferation.

INSULIN RESISTANCE:

Linking insulin resistance many evidences suggest that different steps of the insulin-signaling pathway might be altered. [27]

Autophosphorilation of receptors is followed by the Tyr phosphorelation of IRs1 however, in AD brains and glaucoma many groups reported increases in p(ser) –IRS 1 instead of p(Tyr) IRS1. [28, 29, 30, 31] The Kapogrgannis lab shows that plasma exosomes from AD patients exhibit higher p Ser –IRS -1 levels and lower p tyr-IRS compared to control subjects.

ET-1 enhances the activity of growth factors like PDGF or VEGF [32] secretion promotes synthesis and secretion of thrombospondin and fibronectin and increases platelet adhesion. [33]

ET_B receptors on endothelium activate a negative feedback mechanism that promotes ET-1 clearance and favors the release of NO and PGI2. **(Figure 03)**

ET1 and NO exert a paracrine regulation on each other. [34,35,36]

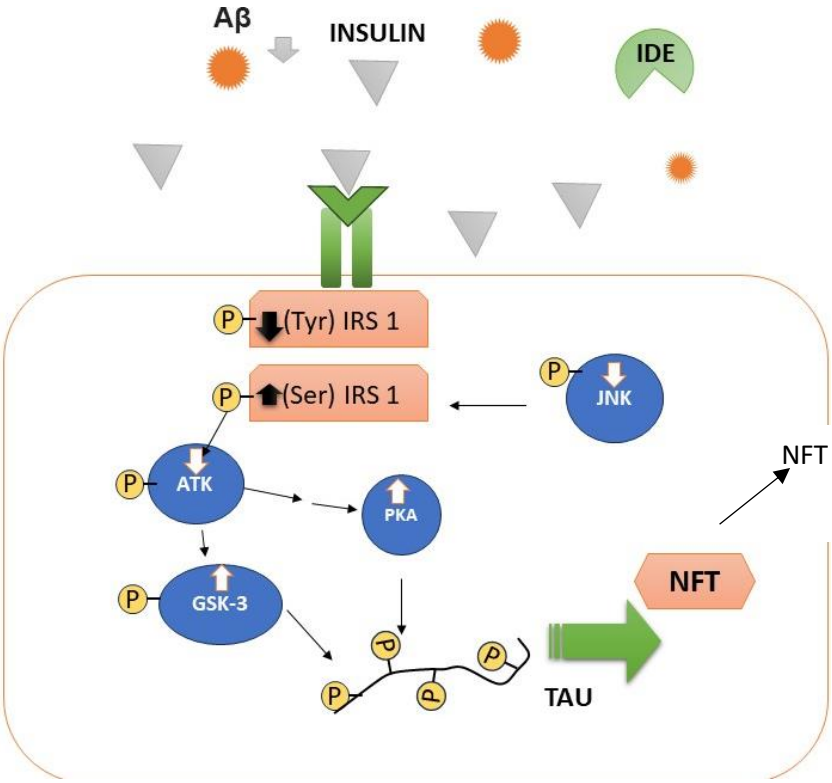


Figure 03: Figure showing Molecular mechanisms and impact of insulin resistance. the increasing of Ser-phosphorylation instead of normal Tyr-phosphorylation affect several downstream of insulin pathway notably increase of TAU proteins and NFT formation

3-improvement of vascular dysfunction by insulin intravitreal injection in glaucoma

Chaoui B.A, 2024, [37] proved in a clinical study the efficacy of intravitreal insulin injection to improve vascular dysfunction in glaucomatous patients. A significant amelioration in the vascular network has been shown in the OCT angiography exam of patients treated with 2 insulin injections within one month between each injection. **(Figure 04.).**

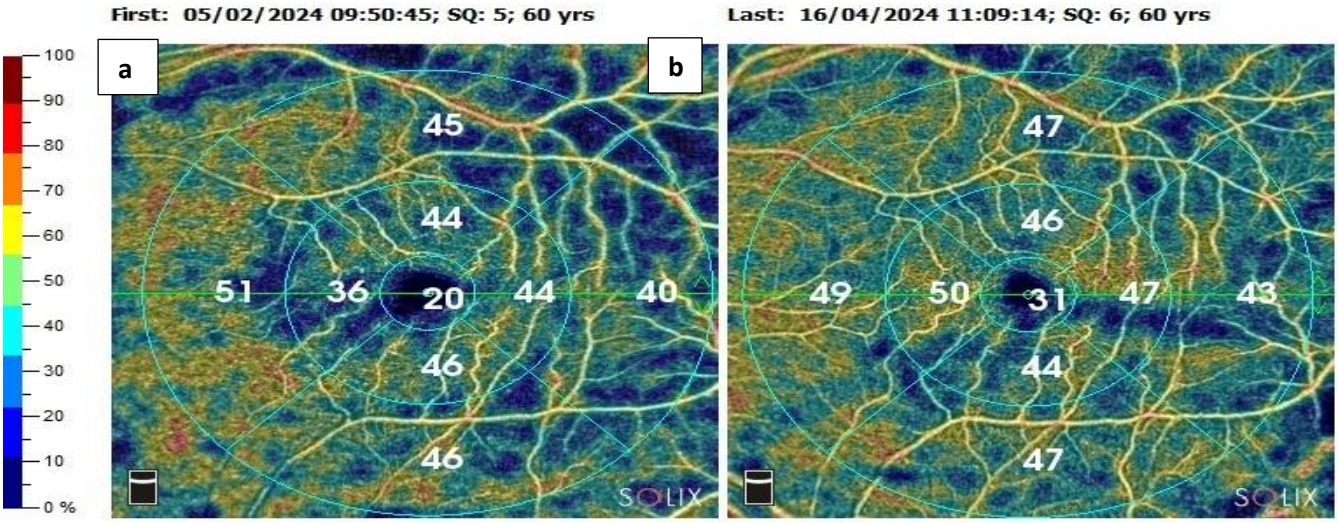
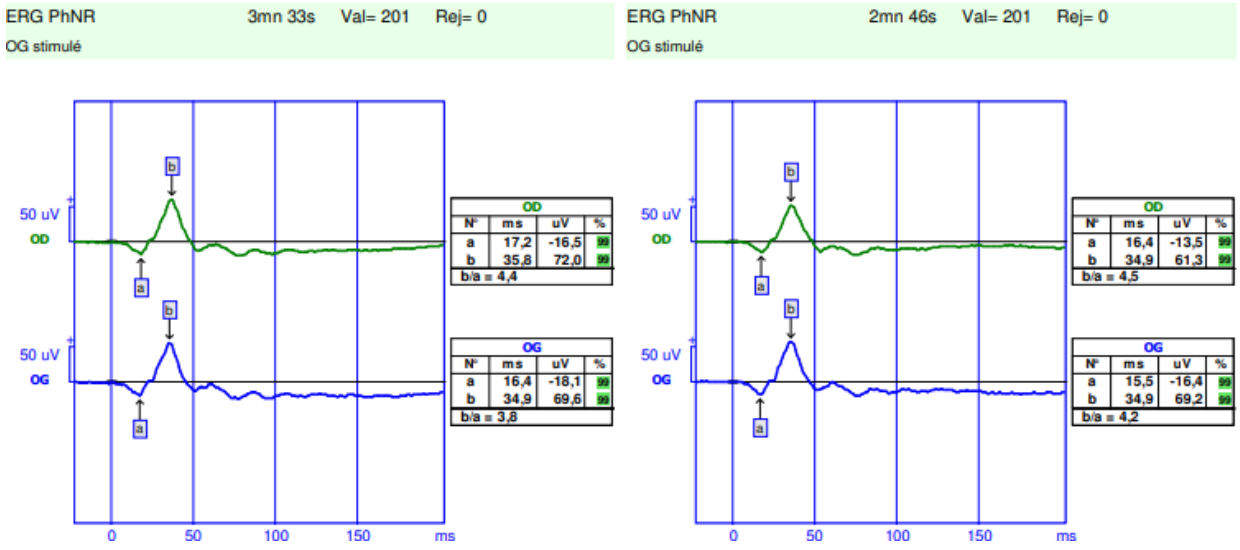


Figure04: Oct angiography exam results before and after treatment with insulin intravitreal injection. **a:** OCT exam before insulin treatment, enhanced vascular density with increased blue zones, **b:** OCT exam results after insulin treatment show decreased blue zones and a significant increase in vascular density.

IMPROVEMENT OF ELECTROPHYSIOLOGY AFTER INSULIN TREATMENT:

Electrophysiological testing of the visual system has been continuously used to involve the evaluation of retinal ganglion cells. This exam was performed with the photopic negative response (PhNR) using ***(metrovision electrophysiology)*** These analyses were based on area under curves (AUC) obtained from receiver-operating characteristics (ROC). **(figure 05)**



Before treatment

After treatment

Figure05: electrophysiology exam results before and after treatment with insulin intravitreal injection. PhNR $_{b/a}$ report shows a significant amelioration after insulin treatment (3.8 before treatment to 4.2 after treatment).

4- Insulin impact on neurodegeneration

Neurodegeneration in glaucoma is characterized by increased cell death abundant neurofibrillary tangles consequent dystrophic neuritis, an impaired deposit of APP (amyloid protein precursor) which result from many pathological pathways such as mTor, beta-amyloid degradation, Tau hyperphosphorylation, and IDE (insulin-degrading enzyme) downregulation.

The expression of apoptotic genes disturbs energy metabolism and causes mitochondrial dysfunction with elevated oxidative stress, causing DNA damage. [38]

There is evidence that insulin had a central origin. The high level of insulin in the brain was consolidated by the detection of insulin secretion in neuron cultures [39], and the presence of insulin immunoreaction inside the Golgi and through the endoplasmic reticulum in the brain. [40] This was detected using insulin antisera.in the retinal layer including the ganglion cell layer.

C -peptide was found in the brain from human cadavers. [41] The synaptic vesicles within the nerve ending store. Insulin in the adult rat's brain on the other hand, the presence of Glut was noticed in the brain likewise, Glut-1 Glut-2 Glut-3, and Glut-4 had different levels and distribution than the peripheral.

Insulin is a part of a family of peptides including insulin-like growth factors I/II (IGF I/II). [42] The insulin receptor is present in the retina. [43] The insulin receptors and IGF-1R (insulin-like growth factor receptor) belong to a family of transmembrane receptor tyrosine kinases permit the kinase of the receptors to phosphorylate these proteins on tyrosine residues. [44] Most of the insulin responses are mediated by IRS1 and IRS2.

Insulin is a potent neuroprotective agent that acts against apoptosis, beta-amyloid toxicity, oxidative stress, and other ischemia. [45] Insulin has an anti-apoptotic effect by mTOR activation which suggests that the P70SK protein one of the downstream targets of PI3K/AKT/mTOR pathway may be one of the mechanisms through which insulin prevents apoptosis. [46]

This activation modulates mitochondrial electron transport chain function and inhibits also Fox1 /HMOX1 and conserves the NAD +/NADH action, which regulates the SIRT1/PGC1α pathway for mitochondrial biogenesis function. [47]

AKT is important to promote neural survival, AKT has been revealed as a primordial mediator of severed aspect of neurite outgrowth implicating elongation, branching, and caliber, more over activated AKT target and inactivates pro-apoptotic proteins such as bad, caspase 9, and glycogen synthetase kinase 3 beta (GSK3). AKT stops apoptosis by preventing the transcriptional activity of p53. [48] Insulin protects against beta-amyloid in the case of insulin resistance which is closely associated with reduced responses to insulin signaling in IR, IRS1, and PI3K signaling. The amyloid beta metabolism is affected as a consequence of tau hyperphosphorylation [49] and the formation of NFT in both AD and glaucoma.

The inflammatory responses are closely associated with the development of insulin resistance by Tumor Necrosis Factor (TNFα)

Obese and AD patients have cerebrospinal fluid insulin (CFS) insulin concentrations lower than in control subjects.

The activity of STAT-3 in astrocytes and microglia depends on glycogen synthase kinase 3 inhibitor (GSK3P) while the inhibition of GSK3 stimulates the production of anti-inflammatory cytokines such as IL and IL10 are decreased pro-inflammatory cytokines such as IL1β, IL6, and TFN-α.

The pathway signaling PI3K/AKT/GSK3 plays an important role in controlling inflammation, the inhibitory effect of insulin on GSK3 activity shows how insulin controls inflammation responses.

NEURODEGENERATION TREATMENT ON GLAUCOMA BY INSULIN INTRAVITREAL INJECTION:
In a comparative clinical study, conducted on 10 patients, after one intravitreal insulin injection compared with placebo group. [37]

The function: was explored by visual field (REODENSTOCK field analyzer device) and visual electrophysiology. An amelioration was noted in average decibel (DB)(+6,62) and average defect (AD) (+1,61) **(Figure 06-07).**

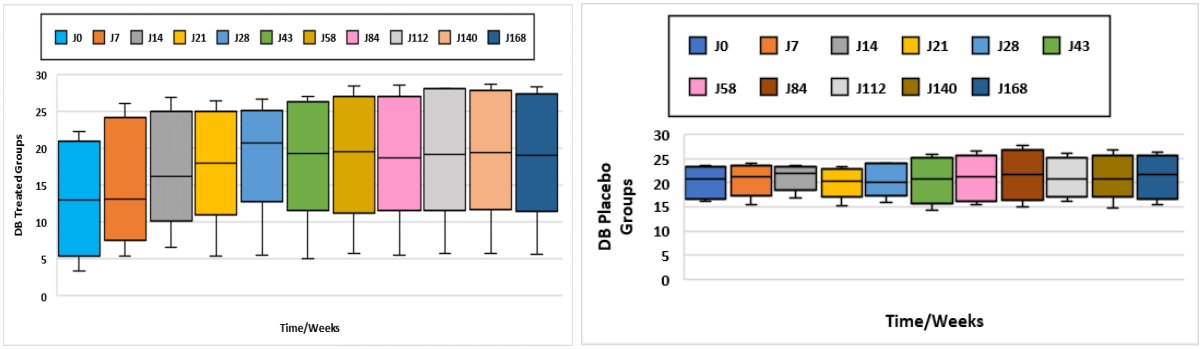


Figure 06: *improvement of average DB in the treated groups during 168 days of follow-up.*

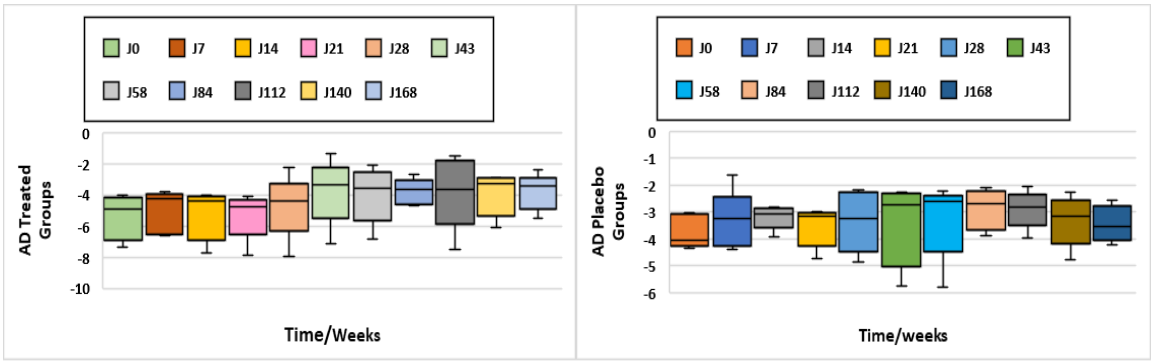


Figure 07: improvement of AD in treated groups during 168 days of follow-up.

The structure: was explored using OCT (OPTOVUE OCT DEVICE)
Improvement was observed in RGC (14 um), and RNFL (11,6 um) results. **(figure 08-09)**

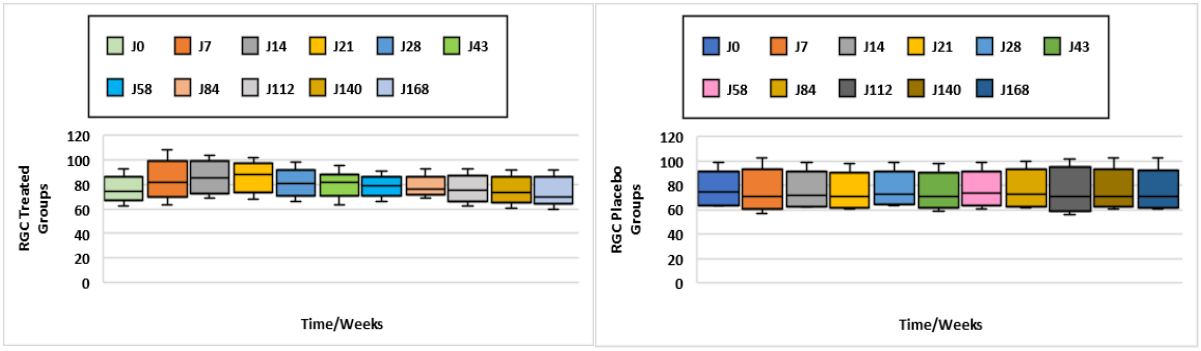


Figure 08: Improvement of RGC in treated groups during 168 days of follow-up.
a: treated group, **b:** placebo group.

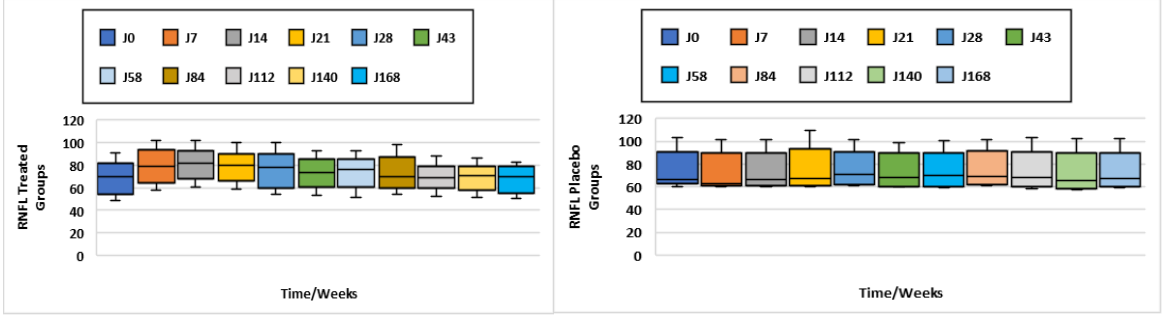


Figure 09: Improvement of RNFL in treated groups during 168 days of follow-up.

On the other hand, structural and functional better amelioration has been noted in 2 insulin injections within one month between each injection subject compared with the one injection group.

Conclusion

Abnormal insulin concentration and /or impaired insulin signaling have a major role in RGC and RNFL survival and also vascular hemostasis in glaucoma.

Insulin is the key to treating glaucoma neurodegeneration and opens a path of research on other neurodegeneration diseases, notably Alzheimer's.

The use of intravitreal insulin injection showed its effectiveness in treating neurodegeneration as well as improving vascular dysfunction.

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