

Effect of Reclining on Retinal Thickness in Diabetes

by

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ABSTRACT

Purpose

Previous studies have shown diurnal variation in retinal thickness in patients with diabetic macular edema (DME) over the course of the day but the cause, presence and magnitude of this variation is controversial. This study will investigate the direct effect of reclining on retinal thickness in diabetic patients with DME using spectral domain optical coherence tomography (SD-OCT).

Methods

Ten patients with DME and proliferative diabetic retinopathy (PDR) or severe non-proliferative diabetic retinopathy (NPDR) (mean age 57.4yrs, SD \pm 6.8yrs) and thirteen healthy controls (mean age 43yrs, SD \pm 13.8yrs) were recruited. Subjects reclined for two hours and retinal thickness using SD-OCT (Heidelberg Spectralis, model spec, Heidelberg, Germany) was measured at five time points; before reclining, immediately after reclining while lying on side, one hour after reclining while lying on side, two hours after reclining while lying on side, and immediately after returning back to an upright position.

Results

Throughout the reclining period, there was a global trend for retinal thickness to change over time within each group ($p=0.068$) and a trend for there to be a greater change in retinal thickness over time in the diabetic group ($p=0.104$). In terms of the change analysis, a significant increase

reached its maximum after two hours of reclining ($+7.2 \pm 13.2 \mu\text{m}$ for the diabetic group compared to $-0.8 \pm 2.4 \mu\text{m}$ for the controls, $p=0.044$). On resuming a sitting position, the retinal thickness significantly reduced in the diabetic group but exhibited some residual increase ($+2.1 \pm 3.2 \mu\text{m}$ for the diabetic group compared to $-0.4 \pm 1.4 \mu\text{m}$ for the controls, $p=0.029$). Of the 6 macular areas / sectors assessed, the temporal macular sector showed the greatest increase in reclining induced DME ($+21.1 \pm 31.1 \mu\text{m}$ for diabetic group compared to $+0.8 \pm 4.2 \mu\text{m}$ for controls, $p= 0.029$)

Conclusion

We found a global trend for retinal thickness of the diabetic group to increase in response to reclining when compared to a control group. This increase was reversed close to baseline values immediately after returning to an upright position. Certain macular areas showed significant increase of retinal thickness, especially the temporal macula. The clinical implications of this reclining induced DME effect are discussed.

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List of Abbreviations

AGE	Advanced Glycosylation End-products
AV	Atrioventricular
BP	Blood Pressure
BRB	Blood-Retina Barrier
CO ₂	Carbon Dioxide
CSDME	Clinically Significant Diabetic Macular Edema
DBP	Diastolic Blood Pressure
DME	Diabetic Macular Edema
EDHF	Endothelium-Derived Hyperpolarizing Factors
ETDRS	Early Treatment Diabetic Retinopathy Study
ET-1	Endothelin-1
HR	Heart Rate
IOP	Intra-Ocular Pressure
IRMA	Intra-Retinal Microvascular Abnormalities
MAP	Mean Arterial Pressure
ml/min	Milliliter per minute
mmHg	Millimeter of mercury
NO	Nitric Oxide
NPDR	Non-Proliferative Diabetic Retinopathy
OCT	Optical Coherence Topography
OPP	Ocular Perfusion Pressure

O ₂	Oxygen
PDR	Proliferative Diabetic Retinopathy
PGI ₂	Prostacyclin
SA	Sinoartial
SD-OCT	Spectral-Domain Optical Coherence Tomography
SBP	Systolic Blood Pressure
TD-OCT	Time-Domain Optical Coherence Tomography
VEGF	Vascular Endothelial Growth Factor

1 Introduction

The prevalence of vision loss in Canada is increasing dramatically with an estimated five Canadians experiencing vision loss every hour¹. Diabetic retinopathy, along with glaucoma, age related macular degeneration, and cataract, is a major cause of vision loss. According to Statistics Canada, there were 1.8 million Canadians with diabetes in 2011², representing an astounding approximately 5% of the population. Vision loss has a profound impact on the individual's quality of life and health, as well as on the cost of health and other support services. In 2007, approximately \$16 billion were spent on vision loss treatment and services in Canada, which amounts to \$500 per citizen. Moreover, there is an additional estimated amount of \$12 billion as a cost of suffering, long term care, special facilities, etc. Vision loss costs are predicted to reach \$30 billion within Canada by the year of 2032¹. However, these figures pale in comparison to, for example, Saudi Arabia where more than 20% of the population have diabetes^{3,4}. All diabetic individuals are susceptible to developing sight threatening diabetic retinopathy, unless this vascular complication is diagnosed and treated earlier. Diabetic retinopathy is a disease that dramatically increases the likelihood of vision loss and it can affect many of the retinal cells. Chronic hyperglycemia in diabetic patients will ultimately elevate vascular endothelial growth factor and, in turn, will result in loss of blood-retinal barrier function and also retinal hypoxia which can lead to several microvascular changes that result in permanent visual loss if not managed properly⁵. Neural changes are also hypothesized to play a role in the development of diabetic retinopathy impacting both the retinal neurons and glial cells. It is not known if the neural changes are induced by vascular changes or induced by diabetic

neuro-pathology and, in turn, whether this initiates the development of vascular changes. Both changes could also be developed concomitantly by separate factors⁶.

One of the key factors in the progression of diabetic retinopathy is the increased permeability of the retinal vascular bed which is thought to be mainly attributed to the development of the blood-retinal barrier dysfunction.

1.1 Retinal blood supply

The retina receives a blood supply through two vascular systems. The outer retina is supplied by the choroid while the inner layers are supplied by the retinal vessels⁶. The retinal blood flow contributes to only 5% of the total ocular blood flow, which is estimated to be 1 ml/min⁷, but it is characterized by a low perfusion rate and high vascular resistance^{6,8}. On the other hand, the choroidal blood flow, which contributes to 65% of oxygen and nutrients supply of the retina, is characterized by a higher perfusion rate and low vascular resistance. The rate of oxygen consumption (i.e. oxygen exchange to the tissues) in the retinal vessels is significantly higher (35%) than in the choroidal vessels (4%).

Both retinal and choroidal vascular systems are supplied by the ophthalmic artery which is branched from the internal carotid artery⁹. The retinal blood flow comes from the central retinal artery which is derived from the ophthalmic artery and enters the retina at the optic nerve to bifurcate into superior and inferior papillary arteries. These papillary arteries are further

branched nasally and temporally to cover the entire inner layers of the retina^{6,9}. The retina drains into the retinal venules to the central retinal vein which exits the eye at the optic nerve and then into the ophthalmic vein. The choroid is supplied by the short-posterior ciliary arteries posteriorly and by the long-posterior ciliary arteries anteriorly, both of which branch from the ophthalmic artery. The choroidal blood supply drains into the vortex veins, with the superior vortex veins draining into the superior ophthalmic vein and the inferior vortex veins draining into the inferior ophthalmic vein. These two veins will exit via the cavernous sinus and will anastomose with the anterior ciliary veins. For the purposes of this thesis, I will now concentrate on a description of the retinal blood vessel and its relevant physiology.

1.2 Retinal Blood Vessels

A blood vessel is composed of three layers. The most inner layer is the intima, which consists of a thin layer of connective tissue enclosed internally by endothelial cells. The intima plays a major role in controlling the permeability of the vessel and the exchange of fluids and nutrients with the surrounding tissues. The outer layer is the adventitia, which consists of a connective tissue and binds the vessel loosely to the tissue around it. The intermediate layer is the media, which contains the smooth muscle cells and bordered internally and externally by the internal and external elastic lamina, respectively. The vascular smooth muscle controls the contractile status of the vessel by contraction or dilation when intravascular pressure is increased or decreased, respectively, in order to maintain the required blood flow and oxygen delivery to the retinal tissues. The structure of the blood vessels, however, is not standardised. Each blood vessel adapts in structure to its own specific role within the vascular tree. Capillaries, for

example, are where most of the exchange of oxygen(O_2), carbon dioxide (CO_2), metabolites, and fluids occur. To make this exchange effective and fast, the walls of the capillaries are thin and numerous consisting of only one layer, the endothelial layer, lined externally by a basement membrane with absence of media and adventitia. On the other hand, the terminal arteries and arterioles, which may exert a high resistance to the blood flow, are larger in diameter and lower in numbers and consist of a relatively thick muscular layer compared to the lumen¹⁰.

The retinal vessels are similar to those of the brain in lacking a fenestration within the endothelial cell wall, whereas the blood vessels of the ciliary body exhibit many cell wall / plasma membrane fenestrations. This specialised structure of the retinal vessels helps to provide tight control of permeability in addition to the greatly reduced number of pinocytotic vesicles, which play an important role in the permeability of blood vessels. The endothelial cells, which are in close communication and coordination with pericytes and glial cells, are tightly joined together to form a “tight” inner retinal-blood barrier, while the outer blood retinal barrier is formed in a similar fashion by the retinal pigment epithelium cells. There are more than 40 proteins thought to be involved in the structure and regulation of these tight junctions between endothelial cells. Some of these proteins are occludin, tricellulin, and the Claudine gene family. However, the exact involvement of each protein and the exact changes that occur to tight junction protein structure in diabetic retinopathy are still to be uncovered⁵.

1.3 Blood pressure (BP)

When the blood is pumped from the heart, it travels through the vascular system to deliver oxygen and nutrients to the body organs and tissues via the arteries and returns to the heart via the veins. The main effective factor of this circulation and movement of blood is the gradient of blood pressure. The left ventricular muscle of the heart contracts and ejects the blood into the aorta with a pressure higher than the atmospheric pressure. After ejection, the ventricle relaxes and then fills up with oxygenated blood again. The blood pressure in the major veins is approximately equal to the atmospheric pressure. The difference between the arterial and venous pressure drives the blood through the vascular system¹⁰.

The blood pressure is usually measured with a sphygmomanometer and described in mmHg units. The blood pressure reaches the maximum point after ventricular contraction and ejection of blood into the aorta. This peak point is called the systolic blood pressure (SBP) and it is normally close to 120 mmHg. The minimum point is reached during relaxation of the ventricle just before the ejection. This trough point is called diastolic blood pressure (DBP) and it is normally close to 80 mmHg. Blood pressure is usually recorded as SBP/ DBP mmHg¹⁰.

1.4 Ocular Perfusion Pressure (OPP)

The blood flow through a vessel is controlled by the inflow blood pressure, the outflow blood pressure, the viscous properties of the blood, and the structure of the specific blood vessel. In the human eye, the blood enters the eye with a blood pressure equivalent to the blood pressure in the

supply arteries. The blood pressure then decreases from these arteries to the capillaries by different resistance factors including the properties of the blood composition (i.e. viscosity), the total length of the arterioles and capillaries, the vascular pattern and the cross sectional area of the vasculature. The venules collect and transfer the blood from the capillaries to the exiting veins. The blood pressure is very low in the veins; however, it has to be higher than intra ocular pressure (IOP) before exiting the eye to permit transport of the blood back to the heart, otherwise the veins would collapse. IOP acts as a compressing force on the vein wall which acts against the blood pressure in the vein itself. If the IOP is higher than the blood pressure in a region of the vein, that region may eventually collapse¹¹. The perfusion pressure in any vasculature is the difference between the arterial blood pressure and the venous blood pressure. The venous blood pressure in the eye almost equals the IOP⁶; the OPP is then defined as the mean arterial pressure MAP (calculated for the eye level since the BP is actually measured from the brachial artery in the arm at the level of the heart) minus the IOP.

The mean OPP is estimated by the following formula:

$$\begin{aligned} \text{Mean OPP} &= \frac{2}{3}(\text{MAP}) - \text{IOP} \\ &= \frac{2}{3} \left(\text{DBP} + \frac{1}{3} (\text{SBP} - \text{DBP}) \right) - \text{IOP} \end{aligned}$$

Where: DBP = Diastolic Blood Pressure

SBP = Systolic Blood Pressure

The factor “2/3” is used to compensate for the blood pressure drop at the eye level with the body erect, since the measurement is actually attained at the level of the heart and the blood column has to overcome the force of gravity to reach the eye^{12,13}.

1.5 Intra-Ocular Pressure (IOP)

The pressure generated by the intraocular components against the outer surfaces of the eye is called the intra-ocular pressure. The main controller of this pressure is the aqueous humor volume while the vitreous humor volume is almost fixed and is not effectively involved in the control of IOP. The aqueous humor in the anterior chamber is produced at the ciliary body in the posterior chamber. Aqueous humor moves from the ciliary body over the surface of the crystalline lens, and then through the pupil to the anterior chamber. Smaller percentage of aqueous humor can also pass by filtration through the vessels of the anterior surface of the iris.¹⁴ The aqueous humour is essential for the oxygen and glucose supply to the avascular lens and cornea. The drainage of the aqueous mainly occurs through the trabecular meshwork and Schlemm's canal, while a smaller volume drains out through the uveoscleral route. The dynamic balance between the production and drainage of the aqueous fluid maintains the normal IOP. The range of normal IOP is between 10-20 mmHg¹⁵

The drainage tissues are thought to regulate the aqueous humour flow in order to maintain a relatively constant level of IOP over long time periods of days to decades as well as over short time periods when the pressure rapidly changes. IOP in the same individual may vary according to different conditions such as body posture, hydration state, time of the measurement, cardiac pulse wave, physical exercise, etc¹⁶.

Several instruments with different techniques are used to measure IOP such as Goldmann applanation tonometer, Perkins tonometer, Tonopen, and air puff tonometry. Goldmann tonometry is considered as the gold standard amongst them.

The Tonopen tonometer was used for the purposes of my thesis study. Tonopen is a small portable handheld instrument with a small measuring tab that measures the IOP on the corneal surface¹⁶. After local anaesthesia is administered, the examiner gently touches the cornea several times with the tip of the tonopen until a consistent IOP reading is obtained and displayed on a small screen. It was thought to be less accurate and less reliable than Goldmann tonometry but later generations of the instrument have shown good correlation with Goldmann tonometry¹⁷ especially within the normal range values¹⁸. In terms of relevance to this study, the portability and smaller size of the tonopen make it more useful for IOP measurement in different body postures or when the patient cannot sit at the slit lamp (Figure 1.1).



Figure 1.1: Tono-Pen XL (Mentor)

1.6 Autoregulation

The blood flow in the retina has to be relatively constant in order to maintain sufficient oxygen and nutrients supply to the retinal tissues. Due to the absence of a functioning sympathetic innervation to the retinal vessels⁹, vascular regulation is achieved by autoregulation which represents an integral adaptive mechanism that maintains the retinal blood flow at a constant level despite changes in the ocular perfusion pressure (OPP). The autoregulatory capacity breaks down at extreme levels of mean arterial pressure and intraocular pressure. These levels are found to be at 30%-40% increase of mean arterial pressure¹³ and at an IOP of 27-30 mm Hg¹⁹.

1.6.1 Myogenic / Pressure autoregulation

Myogenic, or pressure, autoregulation is a response that is mediated by myogenic mechanisms, that is stretch receptors in the smooth muscle cells of the retinal blood vessel walls. Myogenic

autoregulation is induced when there is a change of intraocular pressure or the mean arterial pressure which may result from regular life activity such as exercises, stress, diurnal variation, reclining, etc . The ocular perfusion pressure is changed and that will cause the retinal vessels to correspondingly constrict or dilate to reciprocally increase or decrease the vascular resistance in order to keep a constant blood flow to the retinal tissues²⁰. An acute elevation of systemic blood pressure such as that induced by isometric exercise will result in increased perfusion pressure and this will be autoregulated by constriction of the retinal arterioles to maintain a constant retinal blood flow.²¹.

1.6.2 Metabolic autoregulation

Metabolic autoregulation occurs when there is an change in the metabolic activities of the retinal tissues that requires an increase or decrease of perfusion to meet the metabolic demands of the retinal tissues. So, unlike pressure autoregulation which maintains a constant flow despite changes in OPP, metabolic autoregulation adaptively changes retinal blood flow in response to metabolic demand. In monkeys, an increased retinal blood flow was noticed in flickering light, which increased the retinal metabolism, compared to blood flow in constant light, which reduced the retinal metabolism²². Metabolic autoregulation, or more accurately vascular reactivity, associated with the change of oxygen and carbon dioxide tension in the retinal blood vessels is also a part of the metabolic response that in turn influences the control of retinal blood flow.

Increased oxygen uptake is found to cause vasoconstriction of the retinal blood vessels²³, while increased levels of systemic carbon dioxide lead to vasodilation^{6,24}.

The endothelial layer of the retinal vessels is thought to play a major role in the regulation of the retinal blood flow by releasing vasoactive factors²⁴⁻²⁶ in response to the retinal need for oxygen, glucose, amino acids and other nutrients and to the change in the perfusion pressure. There are more than 10 of these vasoactive factors that have been proposed to play a regulatory role. The most important among them belong to one of two general groups; the endothelium-derived relaxing factors (nitric oxide, prostacyclin, endothelium-derived hyperpolarizing factor) and the endothelium-derived contracting factors (endothelin-1, cyclooxygenase products).

1.6.3 Nitric oxide (NO)

NO is an extremely short half-life potent vasodilator that is constantly released in the retinal vessels to keep the basal blood flow at a constant level^{26,27}. The calcium dependant enzyme NO synthase converts the amino acid L-arginine into L-citrulline to form NO²¹. The endothelial cells usually release NO when stimulated by platelet-derived product (adenosine diphosphate, thrombin), hormones and autacoids (acetylcholine, bradykinin, histamine, noradrenalin), and NO is also released in response to excessive shear stress on the blood vessel wall. By combining with soluble guanylyl cyclase, NO in the smooth muscle cells and pericytes forms cMPG (cyclic guanosine 3', 5'-monophosphate) which, in turn, leads to relaxation of the smooth muscle cells^{26,28}.

1.6.4 Prostacyclin (PGI₂)

PGI₂ is another vasodilator that is released by the endothelium to act in conjunction with NO. It is formed via the action of cyclooxygenase from arachidonic acid. PGI₂ activates cyclic adenosine 3', 5'-monophosphate (cAMP) and leads to relaxation of smooth muscle cells^{26,28}. PGI₂ also helps in the protection from vasospasm, ischemia, and thrombus formation because of its inhibitor effect on platelet function²⁸.

1.6.5 Endothelium-Derived Hyperpolarizing Factors (EDHF)

EDHF are putative substances thought to motivate hyperpolarisation of vascular smooth muscles and pericytes thereby inducing dilation. Unlike NO and PGI₂ which are more involved in the control of large vessels, EDHFs are more associated with smaller vessels and microvessels and thus they play a major role in the peripheral vascular resistance. Some of the factors that are thought to act as an EDHF are: eoxyeicosatrienoic acid (EET), hydrogen peroxide, potassium efflux, and gap junction communication between endothelial cells and smooth muscle cells²⁶.

1.6.6 Endothelin-1 (ET-1)

ET-1 is a potent vasoconstrictor²⁸ that binds with its receptors (ET_A, and ET_B) at the smooth muscle cells and pericytes to cause vasoconstriction. It also may have a vasodilator effect when present in very low concentration. So, ET-1 is thought to play an important role as a local regulator²⁶.

1.6.7 Cyclooxygenase Products

Cyclooxygenase Products are vasoconstrictive substances found in endothelial cells and platelets. The most effective products are thromboxane, prostaglandin, and lipid peroxides²⁶.

1.7 Diabetic Retinopathy

The morphological changes of diabetic retinopathy includes many vascular and microvascular changes, such as the thickening of vascular endothelial basement membrane and eventual endothelial cell loss, loss of pericytes closely associated with the vascular endothelium, vascular smooth muscle cell loss, microaneurysms and capillary closure. These changes lead to break down of blood-retinal barrier and increase in the vascular permeability and leakage of fluids and plasma into the adjacent retinal tissues causing the formation of retinal edema especially at the macula^{5,6}. Vascular Endothelial Growth Factor (VEGF) upregulation, secondary to Advanced Glycosylation End-products (AGE) formation, is thought to initiate loss of integrity of the blood-retinal barrier and the clinical presentation of diabetic macular edema (DME).

Other changes that also play a major role in the development of diabetic retinopathy are intraretinal hemorrhage and venous beading which reflect the progression of capillary closure that leads to ischemia and hypoxia⁵. With the presence of ischemia, the hypoxic retinal tissues further up-regulate angiogenic growth factors (especially VEGF)⁶. This ischemic driven upregulation of angiogenic growth factors is thought to give rise to re-canalization and formation of neovascularization⁵.

1.8 Classification of Diabetic Retinopathy

Diabetic retinopathy is generally classified into nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). NPDR is well defined by the presence of microaneurysms, intraretinal hemorrhage, hard exudates, cotton wool spots, intraretinal microvascular abnormalities (IRMA), and venous beading. The more advanced stage of diabetic retinopathy, PDR, is characterized by the occurrence of neovascularization and the sequelae of preretinal hemorrhage and retinal detachment. Both NPDR and PDR may be accompanied by diabetic macular edema (DME) at any stage⁶.

A severity scale for diabetic retinopathy has been developed based on the Early Treatment Diabetic Retinopathy Study (ETDRS) and the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR). It consists of six major stages (no clinically apparent retinopathy, mild-to-moderate NPDR, moderate-to-severe NPDR, severe NPDR, very severe NPDR, and PDR, with the risk of sight loss dramatically increasing with progression through these groups). DME is classified as clinically significant diabetic macular edema (CSDME) when it threatens the fovea and therefore central visual acuity. According to the ETDRS, macular edema is considered as clinically significant macular edema if one of the following three features were present:

- 1) thickening of the retina at or within 500 μ m of the center of the macula.
- 2) hard exudates at or within 500 μ m of the center of the macula and associated with thickening of the adjacent retina.

3) a zone or zones of retinal thickening 1 disc area or larger, any part of which is within 1 disc diameter of the center of the macula.⁵

Clinically, biomicroscopic examination of the retina with a dilated pupil using a contact lenses is the gold standard method to detect the presence of DME. However, this method is highly dependent on the examiner experience, patient cooperation, ocular opacities, and the volume and extent of the DME. These factors make it a challenge sometimes to precisely detect the presence of DME, especially with mild cases. On the other hand, Optical Coherence Tomography (OCT) objectively takes images of the different layers of the retina with less dependence on the previous factors. Mild DME was found to be more precisely detected by OCT compared to the biomicroscopic examination.²⁹

1.9 Autoregulation / Vascular Reactivity in Diabetes

Evidence suggests that the autoregulatory response is reduced in diabetic patients³⁰⁻³³. Related work has also shown that retinal arteriolar vascular reactivity to isocapnic hyperoxic provocation, in effect an indirect indicator of autoregulatory capacity, is impaired in type 2 diabetes and this impairment is related to the magnitude of DME^{34,35} and to the severity of diabetic retinopathy³⁶.

1.10 Effect of Reclining on Retinal Blood Flow

In order to keep oxygen and other essential elements at an optimal level, blood flow in the healthy retina has to remain relatively constant²⁰. This is achieved by changing the peripheral vascular resistance accordingly in response to the moderate changes in transmural pressure (i.e. a myogenically driven response) exerted across the blood vessel walls. Physiologically, adopting a recumbent position is followed by increased retinal blood velocity and then vasoconstriction to oppose the increased ocular perfusion pressure (OPP), i.e. autoregulation, in order to return retinal blood flow back to baseline values. This response occurs as a result of the increase in OPP that occurs due to the change in the position of the eye relative to the heart when reclining.

1.11 The Role of the Vagus Nerve

The activities of the human body are controlled by the somatic motor nervous system and the autonomic nervous system. The somatic motor nervous system deals with the adjustment to the external environments by innervating the voluntary skeletal and striated muscles, while the autonomic nervous system controls the internal activities that are needed to maintain stable internal status by innervating the involuntary smooth muscles. The two systems interact to respond to the needed activities of the human body³⁷.

The autonomic nervous system consists of three major parts, sympathetic, parasympathetic, and enteric systems. Functionally, the sympathetic system controls the activities that are activated by the human body in response to stress and abnormal circumstances like heart beat acceleration and increase of blood sugar level, while the parasympathetic system acts to maintain a stable

normal level of resources that the body needs. The enteric system controls the gut functioning. Anatomically, the pre-ganglionic fibres of the sympathetic system leave the nervous system at all thoracic levels of the spinal cord plus L1 and L2 levels, while in the parasympathetic system the pre-ganglionic fibres emerge along with the III, VII, IX, and X cranial nerves and at the levels of S2, S3, and S4 of the spinal cord. The main neurotransmitter released by the postganglionic fibres of the sympathetic system is noradrenalin, while acetylcholine is released in the postganglionic parasympathetic system³⁸.

The vagus nerve is the tenth cranial nerve which travels from the brain to the thoracic and abdominal areas of the human body and that is why it is called vagus which means “wandering” in Latin. It is mostly composed of sensory fibres³⁹ and contains three quarters of the entire parasympathetic nerve fibres⁴⁰. Physiologically, the vagus nerves mainly represent the parasympathetic nervous system^{38,40}. The vagus nerve leaves the brain at the medulla and innervates the heart, lungs, oesophagus, stomach, small intestine and many other visceral organs⁴¹.

One of the main functions of the vagus nerve is the control of heart rate in combination with the sympathetic system which plays the major role in the blood circulation. Stimulation of the vagus nerve reduces the cardiac output that may reach zero with strong stimulation, while sympathetic stimulation may double the cardiac output. The two systems are stimulated reciprocally, i.e. when the sympathetic system is stimulated, the parasympathetic is inhibited and vice versa. The vagus nerve synapse at the sinoartial (SA) node and the atrioventricular (AV) node. It also

innervates the atrial muscle and, with much less effectivity, the ventricles and this distribution limits the potency of the vagus nerve to mainly reduce the heart rate with less effect on the contractions⁴¹. The stimulation of the vagus nerve is regulated by the nucleus tractus solitarius (NTS) in the medulla. When the arterial blood pressure changes, the medulla receives signals from specialized receptors (baroreceptors) that are fired or inhibited when the arterial walls stretch or relax due to increased or decreased arterial pressure. These baroreceptors are concentrated mainly in two locations, the carotid sinus and the aortic arch and are innervated by glossopharyngeal nerve and aortic nerve (which in turn combines with the vagus nerve), respectively. At the medulla, the stimulation of the sympathetic system and the vagus nerve is regulated according to the received alerts from baroreceptors. If stimulation of the vagal nerve occurs, the sympathetic stimulation is inhibited and vice versa⁴².

An example of this regulation is when the body posture changes from the supine to the erect position; the arterial pressure will decrease in the head and thoracic parts of the body which cause increase in the firing of the baroreceptors at the carotid sinus leading to strong sympathetic stimulation and vagal inhibition to maintain the blood flow to the brain and upper body parts to prevent fainting⁴¹.

There is no evidence of a direct effect of the vagus nerve on the retinal blood flow as there are no autonomic innervations to the retinal vessels. The vagus nerve, however, may have an indirect effect by changing the entire arterial blood pressure which consequently changes the ocular perfusion pressure to the retina especially during stress conditions like changing the body

position. Chasidy and co-workers⁴³ found a significant drop in mean ocular perfusion pressure after changing body position from supine to standing in patients with autonomic failure and patients with baroreflex failure.

On the other hand, the retinal vessels are found to have cholinergic and adrenergic receptors^{22,43} which raises questions about the role / function of these receptors. The autoregulation of the retinal blood flow has been investigated thoroughly especially over the last two or three decades. With the development of new research tools especially advanced ocular imaging, there might be a need to reconsider research on the possible presence of an autonomic role in retinal blood flow regulation, the nature and role of the autonomic receptors found in the retina, the substances that may bind with these receptors, and the effect of vagal nerve stimulation therapy on retinal blood flow.

1.12 Optical Coherence Tomography (OCT)

Optical Coherence Tomography (OCT) is an imaging technique originally developed in 1991⁴⁴ to scan the posterior structures of the eye and produce a high resolution cross-sectional image of the retinal structures as far back as the choroid. The principle of operation of OCT is similar to conventional ultrasound imaging, but instead of recording a pulsed sound echo, OCT detects the intensity and time delay of light, back-reflected from various features within the imaged object resulting from spatial variations of the tissue refractive index. Original versions of OCT measured the time needed for light to be reflected and scattered from the different layers of the retina and compared the time delay to a reference beam of light. The time difference between the

reflected and reference beams is translated by software into a two-dimensional cross-sectional image. Time domain OCT (TD-OCT) requires scanning of the reference mirror, which limits the speed of data acquisition to less than 15 KHz and thus increases the probability of motion artefacts in the scan. Furthermore, the signal-to-noise ratio of TD-OCT is inversely related to the frequency of the reference mirror.

Spectral-Domain OCT (SD-OCT) is a new generation of OCT which requires no scanning of a reference mirror. Instead, SD-OCT necessitates wavelength scanning of the optical beam either at the input or the output of the OCT interferometer. The reference light in SD-OCT interferes with the light reflected from the retina and is processed by a spectrometer that measures the reflected light simultaneously enabling for a larger amount of data compared to the sequential measurement in TD-OCT. This data is then transformed into an image in depth. So, by scanning a laser beam across the retina, the SD-OCT system analyses the interference pattern and produces depth information of the retinal layers⁴⁵.

One of the major differences between the two OCT techniques is that TD-OCT takes six radial scans and interpolates the areas between these six scans, while the SD-OCT consists of more than 65000 scans arranged using a standardized x, y spacing in a 6 mm area resulting in a much better spatial resolution and an improved representation of macula morphology, thereby reducing the chance of missing any pathology. SD-OCT has a higher axial resolution and acquisition speed compared to the older Time-Domain OCT (TD-OCT). This higher axial resolution (up to 3 μ m for SD-OCT vs. 10-15 μ m for TD-OCT) and higher acquisition speed (0.07 seconds for SD-

OCT vs. 1.7 seconds for TD-OCT) has improved the signal-to-noise ratio and reduced the effect of eye movements. Due to the faster processing capabilities, SD-OCT also allows for a true 3-D scanning of the retinal tissues. Although both SD-OCT and TD-OCT systems are reliable and repeatable⁴⁶⁻⁴⁹, the clear advantages of SD-OCT in terms of improved spatial and axial resolution provide the opportunity to detect and evaluate previously irresolvable detail and subtle changes in morphology associated with retinal diseases.

During the last decade, OCT has been widely used as a valuable tool in the evaluation and diagnosis of diabetic retinopathy. It provides structural and quantitative information of the eye which was previously impossible using the traditional procedures of ocular examination, fundus photography, and Fluorescein angiography.

For the purpose of my study, I will use the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) (Figure 1.2) which has a unique laser tracking system that improves the repeatability and reproducibility of the device. The reference laser beam tracks the eye using point-to-point registration and directs the scanning beam to the same position every time the images are taken^{50,51}.



Figure 1.2: Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany)

1.13 Diurnal / Nocturnal Variation in DME

A number of studies have investigated the diurnal and nocturnal variation in diabetic macular edema⁵²⁻⁵⁶ with some of them suggesting a possible role of the body posture^{52,55} but none of these studies have directly investigated the effect of body posture on retinal thickness. Even for the studies that included reclining of the participants in their protocol^{52,55}, all the measurements were actually taken in the upright sitting position for the purpose of OCT measurement which may lead to missing a possible effect of reclining on the retinal thickness. In addition, most of the previous studies were conducted before the development of the new generation of SD-OCT^{52,55,56}.

1.14 Summary

In summary, the unique retinal vasculature is a part of the vascular system that is affected by changes of the blood pressure which is an important factor, along with IOP, in the control of the OPP. Any change in OPP that may result from changes of blood pressure or IOP is regulated in the retinal vessels by autoregulation to maintain a relatively constant blood supply to the retinal tissues. This autoregulatory response is essential to maintain a constant level of retinal blood flow when the mean arterial blood changes during daily activities such as reclining. When the human body reclines from the upright position, OPP is increased due to changing the position of the eye relative to the heart. In the normal retina, this increase of OPP is opposed by vasoconstriction to maintain the retinal blood flow constant. In patients with diabetic retinopathy, autoregulation / vascular reactivity is found to be impaired and this impairment is related to the concomitant severity of diabetic retinopathy and the magnitude of DME. In the last two decades, OCT was introduced as a valuable imaging device that allowed for better diagnosis and evaluation of retinal diseases, such as diabetic retinopathy, and the morphological changes that underlie it. A new version of OCT, that is SD-OCT, provides a higher resolution and faster acquisition time than TD-OCT which, in turn, helps by obtaining objective imaging of the retina with precise quantitative values especially in cases with DME.

2 Rationale, Hypotheses and Aims

2.1 Rationale

Retinal blood flow is autoregulated by the retinal resistance vessels that adapt to any changes in the ocular perfusion pressure in order to maintain blood flow at constant levels. Many studies, however, have shown that autoregulation and vascular reactivity is disturbed in diabetic patients³⁰⁻³³. This disturbance of retinal blood flow in patients with diabetes will result in insufficient vaso-constriction on changing from an up-right to a supine position and, in turn, will exaggerate the increase in OPP resulting from body posture change.

The morphological retinal changes of diabetic retinopathy lead to breakdown of the blood-retina barrier. The compromised integrity of the blood-retina barrier will increase the permeability of the retinal vessels in patients with diabetes and, thus, will increase the leakage of serum components into the adjacent retinal tissues. This leakage of fluid across the blood-retina barrier into the retina is thought to be especially prominent when the perfusion pressure increases and consequently will increase the retinal thickness resulting in DME. Therefore, OPP manipulation can be used to modulate retinal thickness which, in turn, can be applied clinically to assess blood-retina barrier integrity. OPP manipulation and the resulting change in retinal thickness can be used as an early indicator of the potential development of diabetic retinopathy and the possible clinical formation of DME.

Increased ocular perfusion pressure can be simulated by adopting a reclining position. Upon reclining, the eyes are lowered to the level of the heart, rather than being 30cm above the heart in the upright position⁵⁷. This change in postural position from upright to supine will result in an increase in OPP.

The use of SD-OCT in this study will significantly enhance the accuracy of the retinal thickness measurement compared to the older generation of TD-OCT resulting in reduced measurement variability and improved confidence to detect change in retinal thickness as a result of change in body posture. In this study, however, retinal thickness measurement for the reclining position will be taken while the participants remain reclined. SD-OCT images will be acquired when the participant is sitting for the upright position, and while the participant is lying on their right side for the reclining position.

Furthermore, this study will use SD-OCT methodology in the form of the Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) for the retinal thickness measurement which, in addition to its accuracy and higher resolution and improved acquisition time, has a unique tracking system will allow the optimal registration of images, and the detection of retinal thickness changes, for the exact same retinal area between the various sets of images across upright and reclining positions. The features of the Spectralis SD-OCT also allowed for studying each sector of the nine ETDRS map while the previous studies were looking mainly at only the global macular and/or the foveal retinal thickness.

2.2 Hypothesis

We hypothesize that retinal thickness will significantly increase in diabetic patients with severe NPDR/PDR and DME following the adoption of a reclining position when compared to control subjects.

When adopting a reclining position, the OPP will increase for both diabetic retinopathy patients and healthy controls. This increase will be opposed by an increase of vascular resistance that reduces the retinal vessel diameter. Vasoconstriction, however, is impaired in diabetic patients and that reduced vasoconstriction response will exaggerate the increase in OPP in diabetic patients and will further enhance the leakage of serum constituents into the adjacent retinal tissues through an already compromised blood-retinal barrier and, hence, increase the retinal thickness particularly in the diabetic patients.

2.3 Aim

The aim of the project is to study the effect, if any, of reclining on retinal thickness in severe NPDR/PDR patients with DME compared to healthy controls using SD-OCT, a new non invasive imaging device that can accurately measure the thickness of the retina. The study defines the direct effect of reclining on the retinal thickness of NPDR/PDR patients compared to that effect in healthy controls.

3 Effect of Reclining on Retinal Thickness in Diabetes

3.1 Introduction

The inner and outer blood-retina barriers (BRB) are established to minimize vascular permeability to the retina and loss of integrity of this barrier plays a major role in the development of diabetic retinopathy and especially diabetic macular edema (DME)⁵. It is generally accepted that inner BRB breakdown is particularly implicated in the pathogenesis of DME^{58,59}, although a role for outer BRB breakdown is also suspected⁶⁰. Despite the evidence of BRB breakdown in diabetic retinopathy and DME being established^{61,62}, the clinical management of DME is still particularly challenging⁶³. Clinical DME assessment utilizes contact lens stereo fundus biomicroscopy in order for a clinician to subjectively recognize thickening of the transparent retina. Consequently, it is problematic to differentiate early retinal thickening from normal variation in retinal thickness⁶⁴ and substantial differences in defining the extent and location of retinal thickening in a given patient have been shown to exist between experienced retinal specialists⁶⁵. These difficulties in the clinical assessment of DME occur especially in environments without advanced imaging techniques such as SD-OCT.

Furthermore, various aspects of BRB physiology remain unexplored, including the influences of diurnal variation, body posture and gravity and these factors will undoubtedly result in increased measurement variability unless properly understood and accounted for, irrespective of the method of DME evaluation and quantification using advanced imaging techniques. Interestingly, the clinical presentation of DME is known to be exacerbated by the relatively short-term elevation of blood pressure and then to reverse after appropriate blood pressure treatment,

suggesting that ocular perfusion pressure (OPP) may be an additional factor determining the presentation of retinal thickening in patients with DME.

Retinal blood flow is maintained at a relatively constant level via an intrinsic autoregulatory mechanism, irrespective of any physiological change in the OPP. Retinal vascular reactivity, however, is known to be disturbed in people with diabetes²⁰ and the magnitude of this impairment is related to the magnitude of DME^{34,35}. Increased OPP can be achieved by reclining patients from an erect to a supine body posture which results in the heart no longer having to pump blood against the force of gravity to the eye and consequently increases the OPP. Since retinal vascular regulation is disturbed in diabetes, this increase in OPP will occur in the presence of a less effective autoregulatory mechanism that normally would drive vasoconstriction to minimize any increase in retinal blood flow in the reclined position.

A few studies have reported a diurnal variation in OCT quantified DME and some have suggested that this effect might be related to body posture induced increase in OPP while reclined. However, none of these studies systematically investigated a time relationship between reclining and DME magnitude and all have objectively measured retinal thickness in the erect position, possibly allowing for at least a partial reversal of retinal thickening. In addition, most of the retinal thickness measurements were performed using TD-OCT which has less axial and spatial resolution, longer acquisition time, and therefore greater variability than the more recent SD-OCT which also allows for accurate tracking and follow up of the same macular area through consecutive measurements.

The development of a non-invasive functional test to challenge BRB function would represent a major step forward in the clinical management of diabetic retinopathy and DME. Such a test would allow the manipulation of the clinical presentation of DME, potentially emphasizing affected retinal areas that otherwise might only exhibit “sub-clinical” DME. It could be used to quantify the efficacy DME treatments including laser and anti-VEGF therapies and would also have major benefits in other retinal vascular diseases. Finally, a fuller understanding of the impact of reclining in patients with DME, and the associated increase in OPP, may drive changes in sleeping behaviour of affected patients so that they sleep propped up, especially post-treatment. We hypothesized that retinal thickness will significantly increase in diabetic patients with DME following the adoption of a supine position.

3.2 Methods

The protocol of the study was approved by the research ethics board of the University Health Network, Toronto, Canada, and the University of Waterloo Office of Research Ethics. Written informed consent was obtained from each participant after explanation of the nature and possible consequences of the study.

3.2.1 Subjects

Two groups were recruited for the study, a healthy controls group (n=13) and a diabetic group with severe NPDR/PDR with macular edema (n=10). Inclusion criteria were age of 23-75 years, log MAR visual acuity of 0.2 or better, and type 1 or 2 diabetes for the diabetic group. Exclusion criteria were distance refractive error of ± 6.00 DS or more and/or ± 2.50 DC, any eye disease or

disorder other than diabetic retinopathy (diabetic retinopathy exemption did not apply to the control group), any clinical history of autonomic neuropathy, and any systemic medication that has a vasoactive effect, except medication that is used to control diabetes or hypertension.

3.2.2 Protocol

Participants were asked to refrain from taking any caffeine-containing drinks or food containing high amounts of saturated fat from the night before the day of the study. Caffeine has an impact on cerebral blood flow and retrobulbar hemodynamics while saturated fat may impair the arterial endothelial function. All participants had remained upright for at least four hours prior to the study. Factors such as gender, time of day of the study, light intensity were kept constant and / or matched across groups. The diabetic participants were examined and diagnosed by experienced retinal specialists at the Retina Clinics of the Department of Ophthalmology and Vision Sciences at the Toronto Western Hospital within a maximum of one week prior to starting the study. Upon arrival, each participant underwent a thorough eye exam and rested in a chair for at least 15 minutes before starting the study. Each participant was asked to recline for two hours and retinal thickness of the left eye was measured using SD-OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany) over this period at five time points; before reclining, immediately after reclining, one hour after reclining, two hours after reclining, and immediately after returning back to upright position. Intraocular pressure (IOP) measurement by applanation tonometer (Tono-pen XL, Mentor), blood pressure (BP) and heart rate (HR) using the average mode (of three readings) in the Omron HEM-907 blood pressure monitor were obtained from each participant at time points 1,3, and 5 (Figure 3.1). Each participant was facing up throughout the

reclining period except for the time of taking the measurement where the participant was rolled onto their right side to facilitate imaging of the left eye. Left eye was chosen to keep the consistency of the protocol and to normalize the other effects that may rise from the differences between the two eyes. Another factor was the difficulty to obtain images of the right eye in the reclining position due to the limitation of the vertical movement of the OCT instrument.

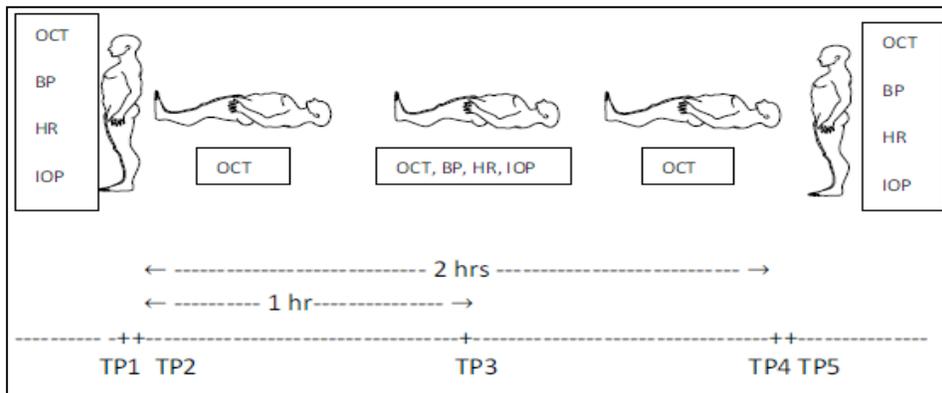


Figure 3.1: Body posture and examination obtained at each time point.(OCT=Optical Coherence Topography, BP=Blood Pressure, HR=Heart Rate, IOP=Intra-ocular Pressure, TP1=time point 1 before reclining, TP2=time point 2 immediately after reclining, TP3=time point 3 after one hour of reclining, TP4=time point 4 after 2 hours of reclining, TP5=time point 5 immediately after return to sitting)

3.2.3 OPP Calculation

From previous studies, mean OPP in the upright position was estimated by the following formula: Mean OPP = $\frac{2}{3} (MAP) - IOP$. That is = $\frac{2}{3} (DBP + \frac{1}{3} (SBP - DBP)) - IOP$, where MAP is the mean Arterial pressure, DBP is the diastolic blood pressure, SBP is the systolic blood pressure, and $\frac{2}{3}$ is a factor used to compensate for the blood pressure drop at the eye level

which is neglected when calculating OPP in reclining position because the eye is almost at the same level of the heart.

3.2.4 Optical Coherence Tomography (OCT)

Retinal thickness within the central 6-mm central area of the retina was measured at each time point by Spectralis SD-OCT using the volume scan mode with 9 frames (9 images are averaged at each measurement) with 25 sections. Sections are 240 μ m apart. The ETDRS map was used to divide the macular area into Central 1mm (C), temporal 1-6mm(T), superior 1-6mm (S), nasal 1-6mm (N), inferior 1-6 mm (I), and the global area (G) which included the entire 6mm macular area as shown in Figure 3.2.

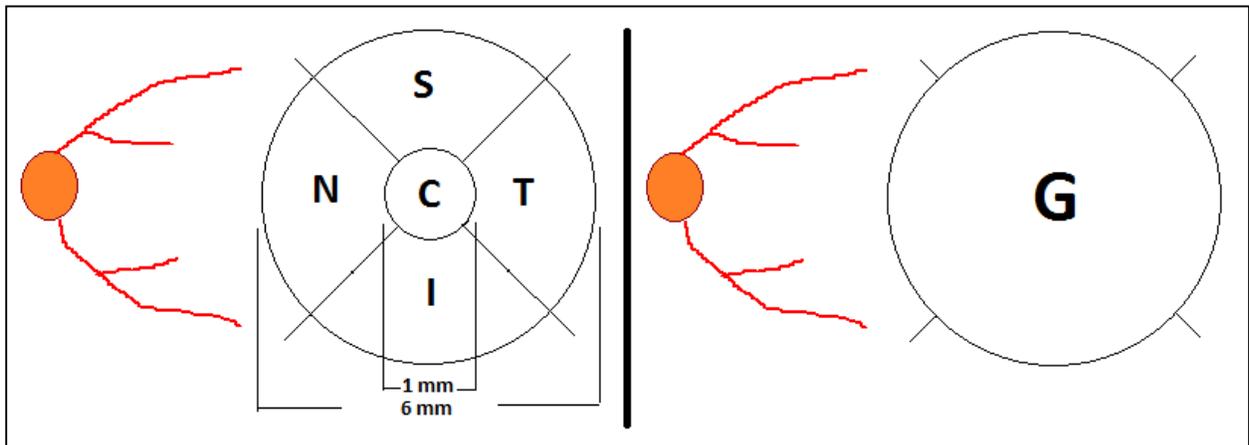


Figure 3.2: Map of the macular area (G= global, C= central, N= nasal, S= superior, T= temporal, I= inferior, the inner fovea-centered circle measures 1mm, the outer fovea-centered circle measures 6 mm)

For the reclining position, the map is rotated approximately 90° counter-clockwise due to changing the head position from upright to reclining on the right side and retinal thickness was recorded according to the area at that position, i.e. temporal area will be superior during reclining but recorded as temporal retinal thickness, and so on (Figure 3.3).

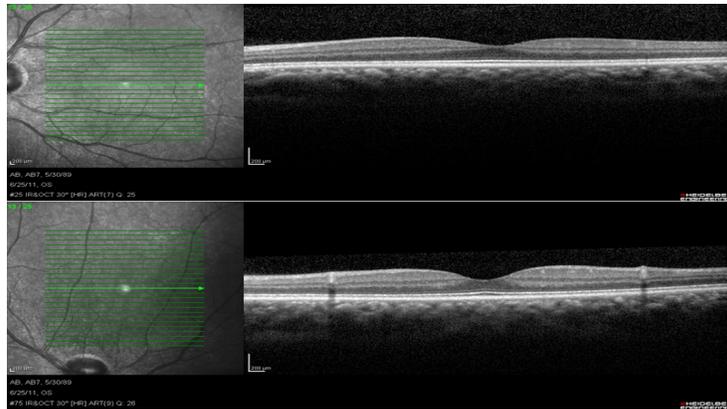


Figure 3.3: OCT image before and after reclining

The TruTrack™ and follow up technologies of the Spectralis OCT allowed for precise alignment and accurate follow up between the images taken at time points 1 and 5 (up-right positions), and between time points 2, 3, and 4 (supine positions).

3.2.5 Statistical Analysis

Data was tested for normality and presented as box and whiskers plots showing change of retinal thickness at each time point in the erect and recumbent position. A Repeated Measures Analysis of Variance (re-ANOVA) was undertaken for the global retinal thickness data considering retinal thickness as an independent variable, and time points as the within-subject factors and group as

the between-subjects factor Analysis of change in retinal thickness was analyzed using One-Way ANOVA to identify any difference in response between the groups.

3.3 Results

Baseline group mean characteristics, and the group mean change in IOP, OPP and MAP over the course of the study are shown in Table 1. Figure 3.4 shows the group mean retinal thickness for each sector of the ETDRS macular grid (left eye) during the test time points.

The results of repeated measures ANOVA showed that the global magnitude of retinal thickness was significantly higher in the Diabetic group ($p=0.027$, Figure 3.4). Although not significant, there was a global trend for retinal thickness to change over time within each group ($p=0.068$) and a trend for there to be a difference in change in retinal thickness over time between the groups ($p=0.104$).

In terms of the change analysis, the change in global retinal thickness (G) reached significance after two hours of reclining ($+7.2 \pm 13.2 \mu\text{m}$ for the diabetic group compared to $-0.8 \pm 2.4 \mu\text{m}$ for the controls, One-Way ANOVA $p=0.044$, Figure 3.5). After returning back to the sitting position, there was a trend for the change in global retinal thickness (G) to return to baseline values in the diabetic group but a significant residual difference between the groups still existed ($+2.1 \pm 3.2 \mu\text{m}$ for diabetic group compared to $-0.4 \pm 1.4 \mu\text{m}$ for the controls, $p=0.029$).

Time	n (female)	Age (yrs)	RT (μ m)	IOP (mmHg)			OPP (mmHg)				MAP (mmHg)		
				baseline	baseline	1hr reclining	Return to sitting	baseline	1hr reclining	Return to sitting	% OPP after 1hr reclining	baseline	1hr reclining
Control n= 13	13 (1 F)	43 (\pm 13.8)	311 (\pm 8)	13.8 (\pm 1.8)	13.9 (\pm 2)	12.8 (\pm 2.2)	45.2 (\pm 6)	65.7 (\pm 9.5)	47.2 (\pm 6.3)	+ 45.4 (\pm 13.7)	88.6 (\pm 10.0)	81.8 (\pm 7.0)	90 (\pm 10.7)
Diabetic n= 10	10 (1 F)	57.4 (\pm 6.8)	348 (\pm 60)	14.4 (\pm 2.3)	15.5 (\pm 2.7)	15 (\pm 3)	52.9 (\pm 11.8)	83.3 (\pm 14)	55.1 (\pm 13)	+ 60.4 (\pm 23.2)	101 (\pm 17.6)	99.2 (\pm 12.7)	105.3 (\pm 17.1)
p value		0.007	0.039	0.482	0.12	0.058	0.055	0.002	0.066	0.066	0.045	<0.001	0.015

Table 3-1: Group mean characteristics of the control and diabetic groups at baseline and the group mean change in intraocular pressure (IOP), Ocular Perfusion Pressure (OPP), and Mean Arterial Pressure(MAP) over the course of the study.(RT= retinal thickness, baseline= before reclining, return to sitting= immediately after returning to sitting position)

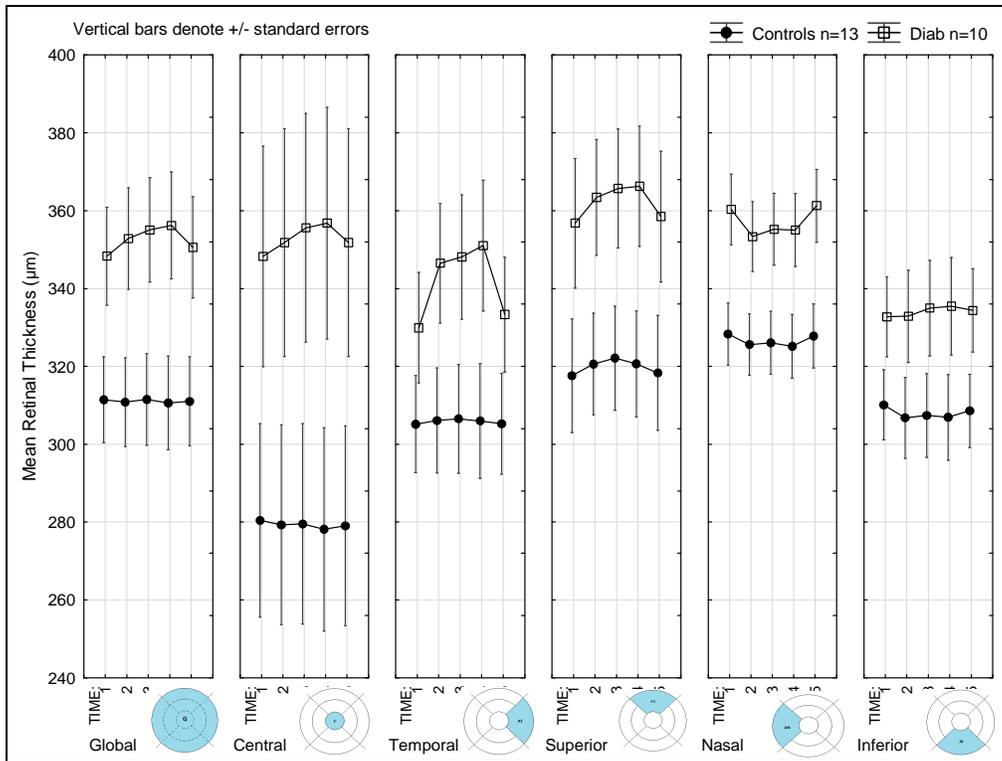


Figure 3.4: Group mean retinal thickness of global, central, temporal, superior, nasal, and inferior sectors of the modified ETDRS grid of the two groups throughout the test time points (1=baseline, 2=immediately upon reclining, 3= after 1 hr of reclining, 4=after 2 hrs of reclining, and 5= immediately after returning back to sitting position)

The change in central retinal thickness (C) was not significant after two hours of reclining but there was a trend for a difference between the groups ($+8.5 \pm 19.7 \mu\text{m}$ for diabetic group and $-2.3 \pm 4.4 \mu\text{m}$ for controls, $p= 0.068$). However, there was a significant difference between the groups in residual increase in retinal thickness in the central area upon returning back to sitting position ($+3.5 \pm 6.8 \mu\text{m}$ for diabetic group compared to $- 1.4 \pm 2.8 \mu\text{m}$ for the controls, $p= 0.030$, Figure 3.6).

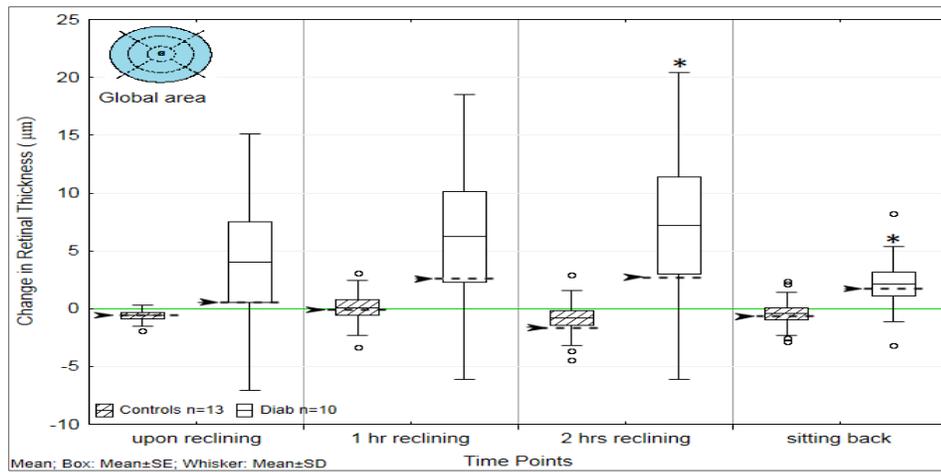


Figure 3.5: Group mean change in retinal thickness of the global macular area (G) over time points. (▶---) indicates the median. (*) shows the significant results between the two groups.

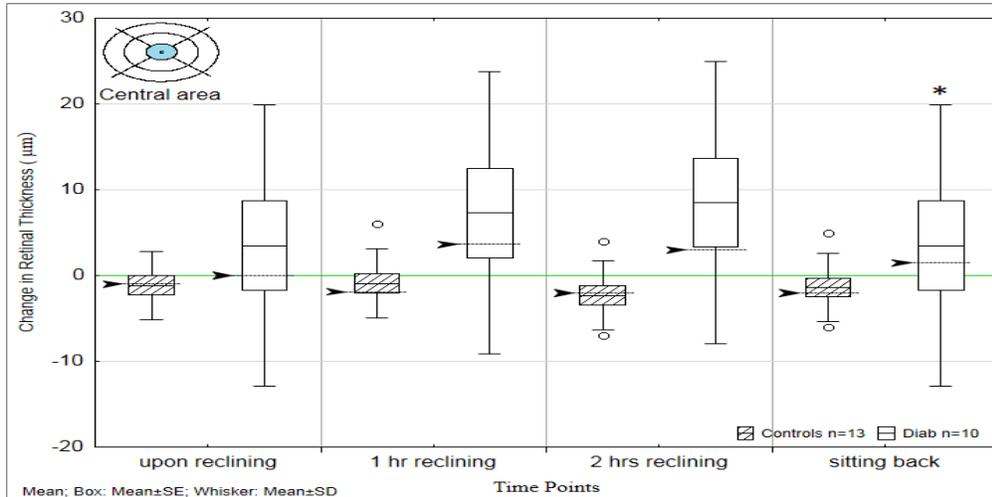


Figure 3.6: Group mean change in retinal thickness of the central macular sector (C) over the time points. (▶---) indicates the median. (*) shows the significant results between the two groups.

The temporal macular sector (T) showed a significant increase in retinal thickness between the groups after one hour of reclining ($+18.2 \pm 28.9 \mu\text{m}$ for diabetic group compared to $+1.4 \pm 3.9 \mu\text{m}$ for controls, $p= 0.049$) and also after two hours of reclining ($+21.1 \pm 31.1 \mu\text{m}$ for diabetic group compared to $+0.8 \pm 4.2 \mu\text{m}$ for controls, $p= 0.029$). Most of the increase in temporal RT resolved upon returning to an upright position but there still was a significant residual increase between the groups ($+3.4 \pm 4.9 \mu\text{m}$ for diabetic group compared to $+0.1 \pm 1.9 \mu\text{m}$ for controls, $p= 0.040$, Figure 3.7).

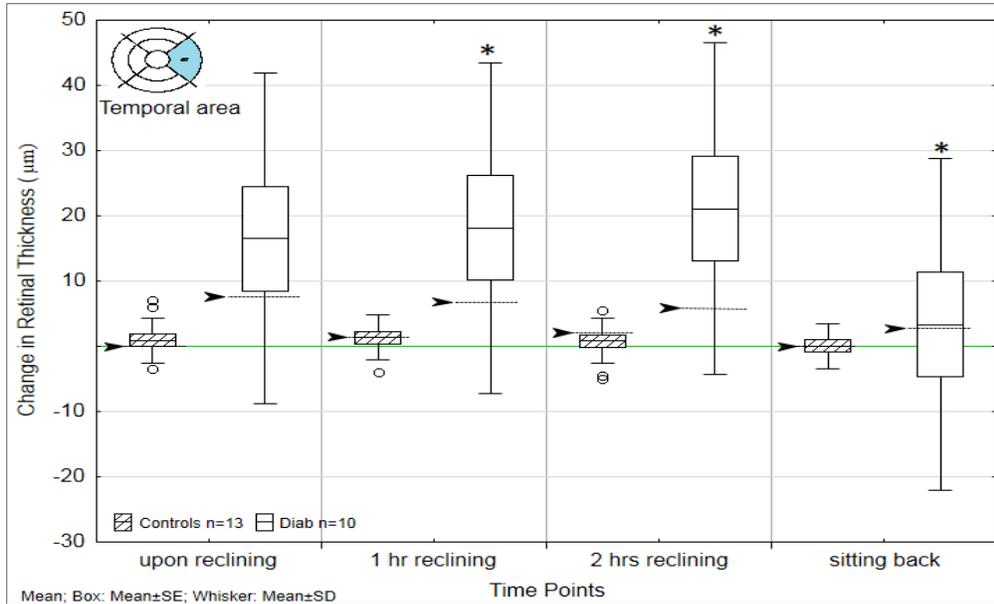


Figure 3.7: Group mean change in retinal thickness of the temporal macular sector (T) over time points. (▶---) indicates the median. (*) shows the significant results between the two groups.

There was no significant change in superior retinal thickness (S) between the groups after two hours of reclining ($+9.5 \pm 20.7 \mu\text{m}$ for diabetic group compared to $+3.0 \pm 3.8 \mu\text{m}$ for controls, $p=0.281$, Figure 3.8).

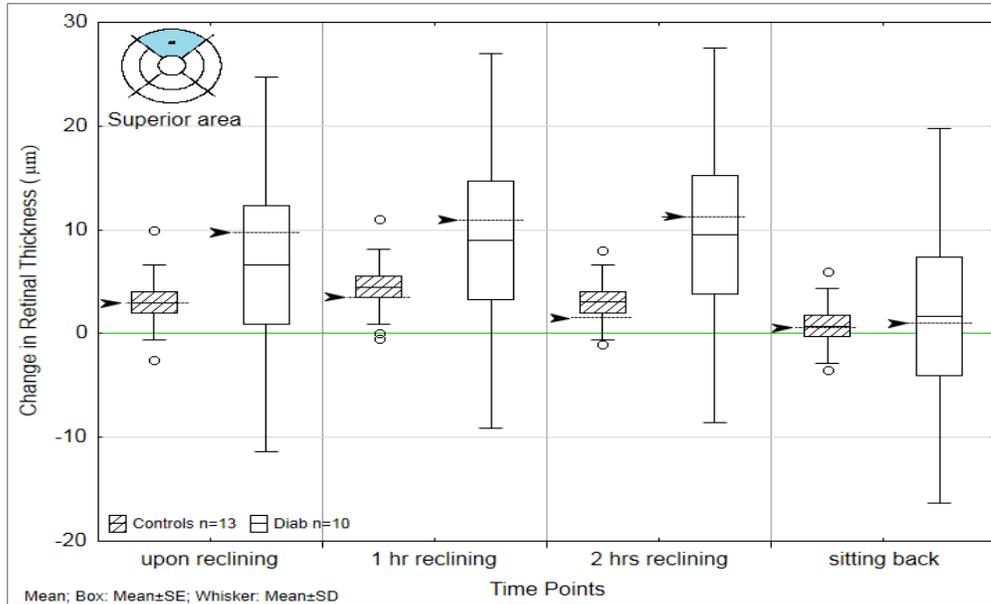


Figure 3.8: Group mean change in retinal thickness of the superior macular sector (S) over time points. (►---) indicates the median. (*) shows the significant results between the two groups.

The nasal sector (N) showed an initial between group difference in change in retinal thickness with the diabetic group showing a significantly greater reduction immediately upon reclining ($-7.0 \pm 5.1 \mu\text{m}$ for diabetic group compared to $-2.7 \pm 2.9 \mu\text{m}$ for controls, $p= 0.018$). The decrease in RT resolved upon returning back to an upright position (Figure 3.9).

The inferior area (I) showed a significant difference in change in retinal thickness between the groups upon returning back to an upright position. The change in retinal thickness increased in the diabetic group while it decreased in the control group ($+1.7 \pm 3.7 \mu\text{m}$ for diabetic group compared to $-1.5 \pm 2.3 \mu\text{m}$ for controls, $p= 0.021$, Figure 3.10).

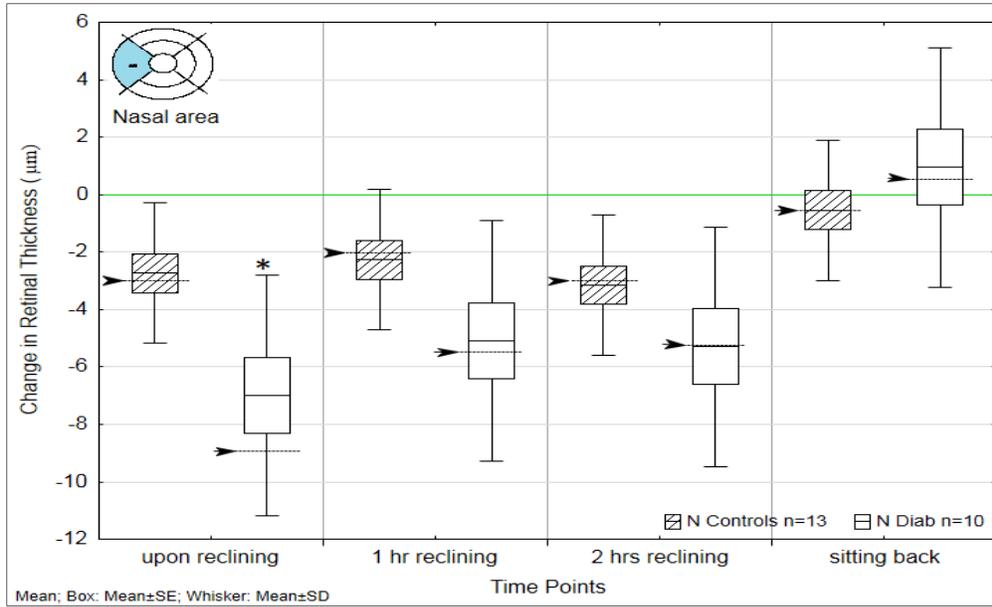


Figure 3.9: Group mean change in retinal thickness of the nasal macular sector (N) over time points. (▶---) indicates the median. (*) shows the significant results between the two groups.

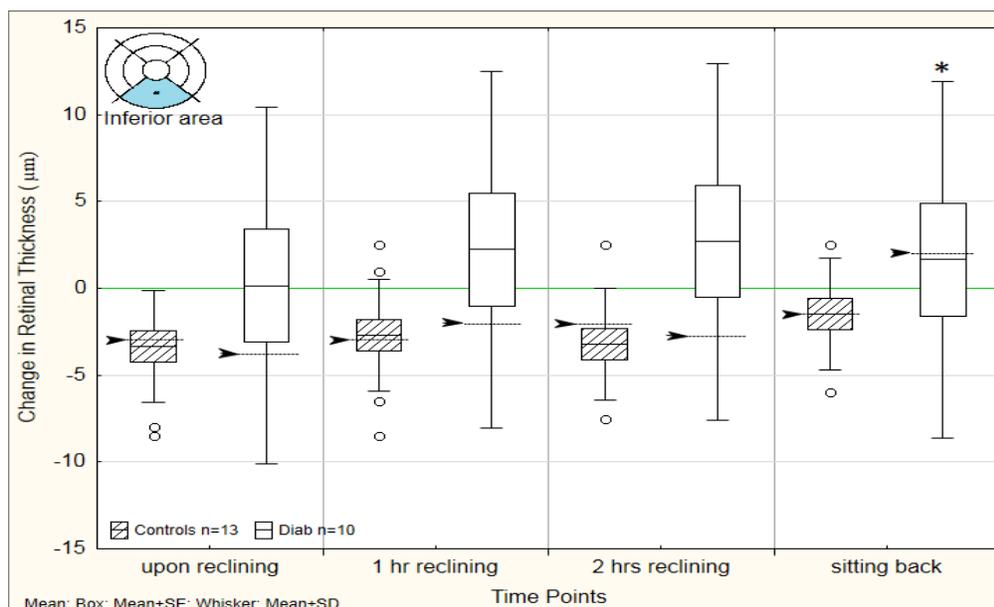


Figure 3.10: Group mean change in retinal thickness of the inferior macular sector (I) over time points. (▶---) indicates the median. (*) shows the significant results between the two groups

The change in group mean ocular perfusion pressure (OPP) in response to reclining was also different between the two groups but was not significant ($+60.4\% \pm 23\%$ for diabetic group compared to $+45.4\% \pm 13.7\%$ for controls, $p= 0.066$). After returning to an upright position, both groups similarly showed a slight increase in OPP compared to baseline ($+4.8 \pm 12.4\%$ for diabetic group and $+4.3 \pm 8.5\%$ for controls, $p= 0.914$).

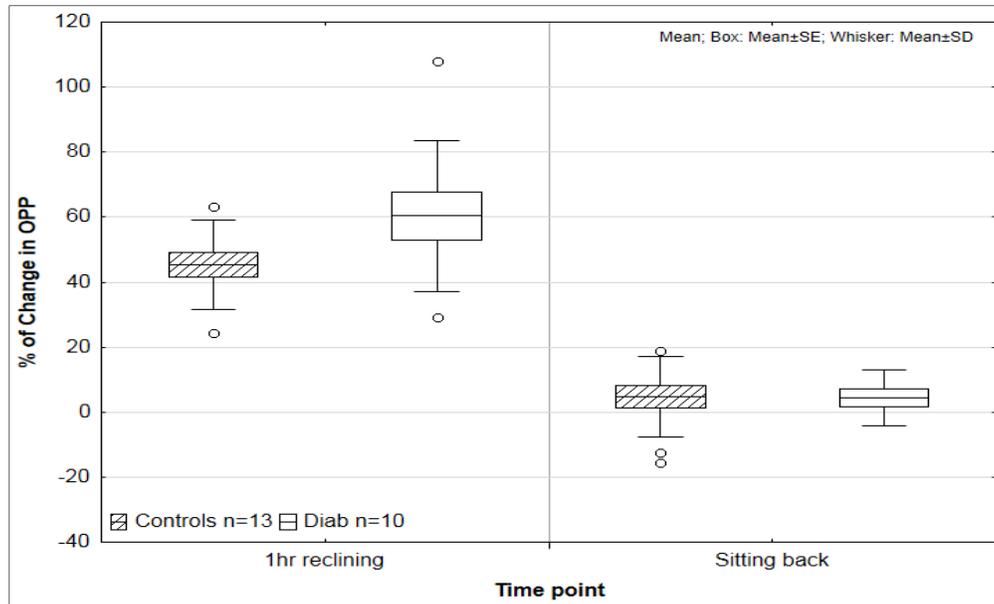


Figure 3.11: Group mean percentage change in OPP from baseline after 1 hour of reclining and after returning back to an upright position.

3.4 Discussion

In this study, which compared the effect of reclining on retinal thickness in the two groups, I found that there was a trend for the mean retinal thickness of the diabetic group to increase in response to reclining, and to return back close to baseline immediately after returning to an upright position. This response was not seen in the control group. The OPP increased in diabetic participants after reclining (60%) more than that in healthy controls (45%) but the difference did not reach a significant level ($p=0.066$). The mean baseline OPP for the diabetic group and the control group was 52.9 and 45.3 mmHg, respectively. In addition, when considering the change in retinal thickness of the diabetic group between the beginning and the end of reclining (TP2 to

TP4), there was a trend for a gradual increase in retinal thickness (Figures: 3.5 to 3.10), suggesting the accumulation of fluids over the time of reclining. Frank and co-workers (2004) studied the diurnal variation in diabetic macular edema and suggested a possible effect of a gravitational factor to explain the decrease of retinal thickness that was found in some of the participants through the course of the day. Polito and co-workers (2006) also found a decreased retinal thickness in clinically significant diabetic macular edema over the course of the day in some of the participants. However, these studies were not directed to the time relationship between body posture and DME, and all the measurements were taken objectively while the participants were in an upright position using a TD-OCT; this may explain why the effect was seen only in some of the participants. In addition, the earlier studies did not consider the change in OPP as a possible factor that may further explain the phenomenon of the diurnal change in RT of people with diabetes. Another study by Larsen and co-workers (2005) found an overnight increase in retinal thickness in fovea-involved diabetic macular edema which was correlated with the change in MAP. Polito and co-workers (2007) suggested that body posture and hydrostatic pressure may play a major role in the formation of CSDME. This study confirms the previous suggestions of a possible gravitational effect and the consequent increase of OPP. In this study, we found a trend for OPP to increase to greater extent for the diabetic group when reclining ($p=0.066$). The reason for this greater increase in the diabetic group could be due to diabetic neuropathy, reduced autoregulatory capacity, higher blood viscosity in the diabetic group, and other reasons that we have not considered. The increased OPP might also be responsible for the slight increase of retinal thickness seen in controls. In a case study, Taibbi and co-workers have shown increased retinal thickness in normal retina after 30 days of bed rest with 6° head-down tilt position. They suggested a role of a "compensatory mechanism" against the increased IOP

that occur after reclining because of the inhibition of choroidal venous drainage and the subsequent expansion against the solid scleral tissue. They, however, did not consider the change in MAP and the consequent change in OPP.

Although there was a trend of change in the global macular area which may be explained by the effect of gravitational shift of blood to the head and the consequent leakage of fluids into the retinal tissues due to the increased OPP, the characteristics of this change vary as a function of position within the macular region. The temporal area showed a prominent increase in retinal thickness while the nasal area showed reduction. The superior and inferior areas showed a slight but not significant increase. Hudson and co-workers (2005) found that relative nasal-temporal asymmetry of macular blood flow is exaggerated in diabetics with CSDME. In this study, there was an interaction between the different sectors when a repeated measures ANOVA test was performed ($p=0.005$ after the Greenhouse-Geiser correction).

Among the ten diabetic participants, four participants did not receive laser and/or injection treatment for the macular edema prior to the study and there was no correlation found between the change in retinal thickness and the time since last treatment was received prior the study. However, the small number of cases and the varied treatments limit this aspect of the study.

There was a correlation between baseline retinal thickness and the change in global retinal thickness of the two groups (i.e. data from both groups pooled) after two hours of reclining ($r = 0.45$, $p=0.033$, and $r^2 = 0.20$). A stronger correlation was found between baseline retinal

thickness and the residual change in central retinal thickness after returning back to upright position ($r = 0.88$, $p = <0.001$) which suggest that the magnitude of the pre-existing edema might be a factor that needs to be considered in future investigations. There was a correlation between the baseline OPP and the change in retinal thickness ($r = 0.56$, $p = 0.005$) for the diabetic and the control group data combined.

The average age of the control group and the diabetic group was 43 ± 13.8 and 57.4 ± 6.8 , respectively ($p = 0.007$). However, age was not found to have an effect on retinal thickness⁶⁶. Furthermore, no correlation was found between age and baseline retinal thickness of the 13 healthy control participants of this study (Figure 3.12).

The rotation of the macular map when adopting a recumbent position and acquiring the SD-OCT images with the participant on their side was a challenge. Participants were instructed to keep their heads as horizontal as possible at the time of the measurement to obtain approximately a 90° counter-clockwise rotation (from the upright position) in order to achieve optimal alignment of the ETDRS grid sectors between the two positions. This was not an issue for the central and global area since they are circular in shape around the fovea which is the point of fixation at the time of measurement. Using a hand-held OCT might be useful in future studies of this reclining induced increase in DME.

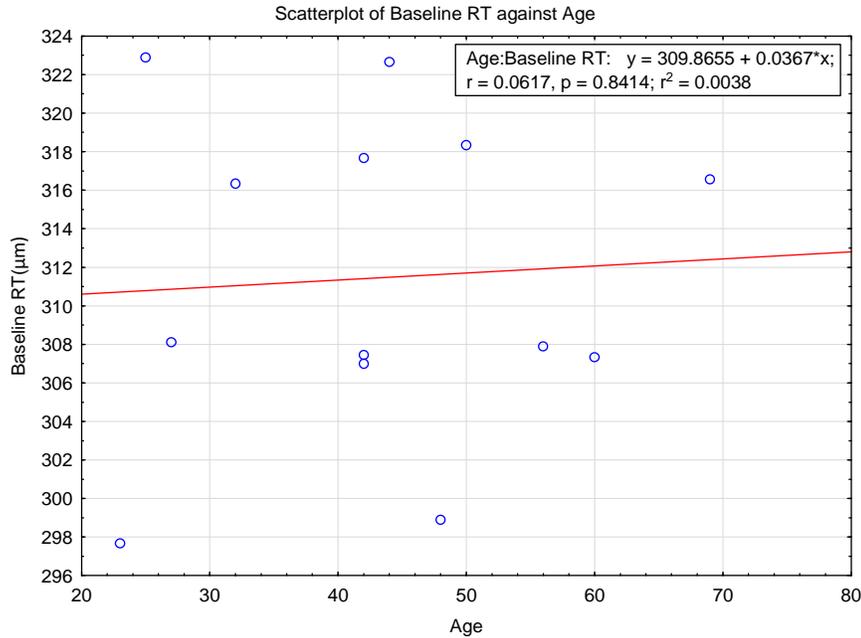


Figure 3.12: Correlation age and baseline retinal thickness in healthy control group

3.5 Conclusion and Future Directions

In conclusion, there was a trend for change in group mean retinal thickness among the diabetic group after reclining for two hours, compared to the normal control group. This effect reversed very quickly upon returning to an upright position. The methodology of this study is anticipated to be developed into a clinical functional test to determine the integrity of the blood retina barrier. Assessment of the blood retina barrier integrity is becoming more relevant to the assessment and treatment of diabetic retinopathy, especially with the introduction of new therapeutic agents which, in turn, may help in the development of early/prophylactic diabetic retinopathy treatments. Further studies might be useful to investigate this effect considering the

different stages of diabetic retinopathy, the location and the severity of the diabetic macular edema, and greater periods of reclining.

4 Discussion

Retinal blood flow is autoregulated, and this autoregulatory mechanism is compromised in diabetes. Understanding the mechanisms that lie behind the disturbance of autoregulation in diabetic retinopathy will be a key factor to better diagnose, manage and treat diabetic retinopathy and the potential formation of macular edema. Autoregulation is mainly a responsive reaction against changes in OPP which induce change in the transmural wall pressure of the retinal arterioles. When the blood pressure increases, autoregulation leads to vasoconstriction of the retinal blood vessels in order to keep the retinal blood flow at a relatively constant level. Reclining of the body is one of the non-invasive methods that could be used to increase the perfusion pressure in the eye. So this methodology could be used as a provocative test to examine the changes in vascular function and inner blood retinal barrier integrity that may occur due to diabetic retinopathy. Reclining induced changes in retinal thickness could be used as a functional indicator of a compromised blood retinal barrier.

A few previous studies have utilized the combination of retinal thickness measurement and body posture to investigate the effect of diurnal and nocturnal variation on retinal thickness in participants with diabetes using the OCT technique. The protocols of the previous studies have included reclining of the participants but the measurements of the retinal thickness, however, were acquired in the upright position which probably will reduce the magnitude of the true effect of reclining induced increase in retinal thickness. In this study, measurements of retinal thickness were taken while the participant was still lying on their side. Contrary to previous work, the specific goal of this study was to investigate the effect of reclining on retinal thickness rather

than to observe diurnal changes of retinal thickness. In addition, most of the previous studies have used the old versions of time-domain OCT while this study was performed using the new spectral-domain OCT which has a higher axial and spatial resolution and shorter acquisition time which helped in reducing the variability between the measurements. Another advantage of applying the spectral-domain OCT in this study was the ability of accurate tracking and follow up of the same macular area through different measurements over time.

The results of this study showed that retinal thickness of the diabetic group tends to increase in response to reclining for 2 hours when compared to the control group. Despite the differences in the protocols and the body posture at the time of measurement, this result effectively agrees with most of the previous studies that investigated the change of retinal thickness over the course of the day. Frank and co-workers (2003) found a reduction in retinal thickness over the course of the day when they investigated the effect of diurnal variation on diabetic macular edema. Polito and co-workers (2006) studied the diurnal variation on CSME and found a similar effect in 7 of the 13 participants. The absence of a significant effect of reclining in the control group in this study also agrees with Frank and co-workers and Polito and co-workers in their previously mentioned studies, although there was evidence of increased retinal thickness in a healthy control after 30 days of bed rest with head tilt in a case study by Taibbi and co-workers. The increased retinal thickness during reclining in diabetic participants in this study was also in agreement with the results found by Larsen and co-workers (2005) who found an overnight increased retinal thickness in participants with fovea-involved diabetic macular edema.

Both of our study groups showed an increased OPP after reclining but there was not a significant difference in the amount of this increase between the two groups. However, the increase in OPP of the two groups after reclining supports some of the previous studies that suggested a possible role of the gravitational effect in the generation of reclining induced retinal edema. In this study, the topographical distribution of the increase in retinal edema during reclining was more pronounced in the temporal retina while the nasal retina showed a decrease in retinal thickness. This can be explained by the effect of the physiological vitreo-retinal attachments at the fovea, optic nerve and ora seratta. When reclining on the right side and imaging the left eye, the optic nerve will be inferior to the fovea. As a result, retinal edema will accumulate temporal to the fovea and nasal to the optic nerve head but this will only be detected in the temporal macula scanned area because the ETDRS map does not extend to the nasal area beyond the optic nerve head. Swelling of the superior and inferior retina will cause stretching of ILM between the fovea and optic nerve head and a resultant thinning of the retina in the nasal scanned area. In addition, it is worth noting that the terminal arterioles and the capillaries temporal to the fovea are the weakest and, therefore, are the most vulnerable vessels to compromised increase in OPP.

The clinical implications of this work are important. The methodology adopted in this study could be applied as a provocative test to earlier detect and exaggerate the presence of retinal edema. This could lead to a more standardized assessment of the magnitude of retinal edema since the effect of diurnal variation would be negated. Furthermore, if this procedure reflects blood retinal barrier integrity, it may be applied to predict the likelihood of treatment outcome in diabetic patients undergoing laser and anti-VEGF treatment; the dose of anti-VEGF drugs may

even be related to the magnitude of reclining provoked retinal edema leading to more precise, predictable and cost effective treatment.

Future work should confirm or deny the potential cause of the reclining provoked increase in retinal edema in diabetic patients. A first step in this respect would be to measure changes in retinal blood flow during prolonged reclining in both health subjects and in people with diabetes. The data in this thesis suggest the possibility of an effect in normal subjects and this should be investigated further since it may have implications for extended work in weightless environments, e.g. astronauts in the international space station. The possibility of generating a shorter protocol should also be investigated using head down reclining slopes and the impact of diabetic retinopathy severity should also be investigated to determine any relationship with reclining provoked retinal edema. Finally, the protocol may have important application in other eye and systemic diseases such as ARMD, glaucoma, stroke, Parkinson's disease, and Alzheimer's disease.

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