Impact of lutein and docosahexaenoic acid-enriched eggs on retina and metabolic health in type 2 diabetes

by

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Abstract

Vision impairment resulting from diabetic retinopathy is a growing concern among individuals with type 2 diabetes mellitus (T2DM). However, early retinal dysfunction can occur even in the absence of clinically detectable diabetic retinopathy. Lutein and docosahexaenoic acid (DHA) are key nutrients to support retina and macular health. Consumption of eggs enriched with lutein and DHA has been shown to influence retinal function in healthy older Caucasian adults positively. This research was planned to examine: i) retinal function and macular pigment levels in individuals with T2DM without diabetic retinopathy ii) discrepancies between self-perceived vision status and objective retinal assessments and iii) the impact of lutein- and DHA-enriched egg consumption on retinal and metabolic health in T2DM. A total of 64 adults (34 T2DM; 30 with normal glucose metabolism, NGM) participated in a cross-sectional study, followed by a 6-week intervention trial involving daily consumption of enriched eggs (0.87 mg lutein, 220 mg DHA). Assessments included electroretinogram (ERG), macular pigment optical density (MPOD), skin carotenoid levels, plasma DHA, glucose and lipid profiles, dietary intake, and vascular and body composition parameters. Individuals with T2DM demonstrated significantly slower ERG responses ($p<0.01$) and lower skin carotenoid scores ($p<0.05$) compared to NGM, despite reporting no perceived vision impairment. Egg intervention increased plasma DHA levels ($p<0.05$) without altering MPOD or ERG responses over 6 weeks. Retinal dysfunction in T2DM may be underrecognized due to discrepancies between perceived and actual vision status. While short-term lutein- and DHA-enriched egg consumption improved DHA status without adverse effects, longer interventions may be needed to influence retinal outcomes in T2DM.

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Abbreviations

AGEs	Advanced glycated end products
ALA	α-Linolenic acid (C18:3n-3)
CCK	Cholecystokinin
CRP	C-reactive protein
DHA	Docosahexaenoic acid (C22:6n-3)
DPA	Docosapentaenoic acid (C22:5n-3; C22:5n-6)
DR	Diabetic retinopathy
ELM	External limiting membrane
EPA	Eicosapentaenoic acid (C20:5n-3)
ERG	Electroretinogram
FA	Fatty acids
ff-ERG	Full-field electroretinogram
FFQ	Food-frequency questionnaire
GCL	Ganglion cell layer
HDL-C	High-density lipoprotein cholesterol
ILM	Inner limiting membrane
INL	Inner nuclear layer
IPL	Inner plexiform layer
LDL-C	Low-density lipoprotein cholesterol
L/Z	Lutein and zeaxanthin
MAGs	Monoacylglycerols
MDA	Malondialdehyde
MP	Macular pigments
MPOD	Macular pigment optical density
NFL	Nerve fiber layer
NPDR	Nonproliferative diabetic retinopathy
OCT	Optical coherence tomography
ONL	Outer nuclear layer
OP	Oscillatory potentials
OPL	Outer plexiform layer
PEL	Pigment epithelium layer
PUFA	Polyunsaturated fatty acid
PWA	Pulse wave analysis
ROS	Reactive oxygen species
RPE	Retinal pigment epithelium
SDA	Stearidonic acid (C18:4n-3)
TAG	Triacylglycerol
TC	Total cholesterol
T2D	Type 2 diabetes
VEGF	Vascular endothelial growth factor

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Chapter 1: Literature Review

Introduction

Diabetic retinopathy is a serious complication of diabetes, caused by prolonged elevated blood glucose levels in the retinal vasculature. The delicate blood vessels in the retina can deteriorate, leading to significant vision loss. Not only does vision loss impede independence, but it also increases one's risk of falls and depression, which can significantly impact quality of life. Diabetes prevalence keeps rising worldwide, and as a result, the incidence of diabetic retinopathy is also rising. Approximately 131 million individuals currently have diabetic retinopathy, and that number is estimated to increase to 205 million by 2045 (Teo et al., 2021).

Dysfunction in the retinal cells precedes any visual symptoms in the retinal vasculature. The early stages of diabetic retinopathy are characterized by the formation of microaneurysms that swell and leak into the retina (Lin et al., 2020). As the disease progresses, hemorrhaging continues, resulting in restricted blood flow, thus promoting neovascularization of new vessels. The most advanced stages occur when the new blood vessels bleed into the vitreous, causing potential vision loss. The gold standard treatment for advanced stages of diabetic retinopathy is photocoagulation therapy, an invasive treatment that cannot cure the disease or reverse vision loss. Considering the limited treatments for this disease, it is imperative to develop easy and accessible strategies to prevent it from happening in the first place.

Extensive research has been conducted on docosahexaenoic acid (DHA, C22:6n-3) and lutein, focusing on their roles in maintaining retinal health and their potential impact on retinal diseases, such as diabetic retinopathy (Lafuente et al., 2021). There is a common agreement that both DHA and lutein play vital roles in maintaining the integrity and health of the retina, indicating that the retina responds to nutrient interventions. However, most of the studies have

examined their individual effects in preventing diabetic retinopathy. There is a notable lack of research on the combined synergistic effect of these two nutrients within a food matrix. In our previous study, we found that healthy older Caucasian adults who consumed two DHA- and lutein-enriched eggs for five days a week for six weeks showed improved retinal function (Walchuk, 2022). Eggs are also an excellent source of choline, essential amino acids, and unsaturated fatty acids, which can inadvertently increase the bioavailability of lutein. Considering the critical role of lutein and DHA in preserving retinal health, it remains to be seen whether lutein- and DHA-enriched eggs can improve the vision of individuals with type 2 diabetes (T2DM).

This review will specifically focus on the retina and diabetic retinopathy, examining the impact of nutrients on retinal health. The aim is to determine whether lutein and DHA-enriched eggs can improve vision in individuals with T2DM as a preventive strategy for diabetic retinopathy.

Retina Anatomy

The retina, with its intricate structure and functionality, lets humans see. The retina is a thin neurosensory tissue composed of seven layers that lines the inner surface of the eye (Kolb et al., 2007). The first layer to form in the retina is the innermost retina pigment epithelium layer (RPE) followed by the photoreceptor layer, the external limiting membrane (ELM), the outer nuclear layer (ONL), the outer plexiform layer (OPL), the inner nuclear layer (INL), the inner plexiform layer (IPL), the ganglion cell layer (GCL), the nerve fiber layer (NFL), and finally the inner limiting membrane (ILM) (Kolb et al., 2007). The nerve cells are located in the innermost (RPE, photoreceptors, ELM, ONL) and outermost layers (GCL), both connected

by synapses (POL, INL, IPL) (Kolb et al., 2007). The retinal layers and their respective components are shown in **Figure 1-1.**

The retinal pigment epithelium (RPE) is a single layer of approximately four to six million epithelial cells found between the outer segments of the photoreceptors and the choroid, which engulfs used-up outer segments (Strauss, 2007). These cuboidal epithelial cells are rich in melanin granules, which function to decrease light scatter and neutralize free radicals and reactive oxygen species (ROS).

The photoreceptor cells are the principal constituent in light absorption. These photoreceptor cells are not equally distributed in the retina, with 120 million rod cells and only 6 million cone cells. Rods consist of the rhodopsin pigment, which is composed of retinal, a derivative of vitamin A, and the protein opsin (Strauss, 2007). Rhodopsin pigments are sensitive to light and are primarily used to enable vision in dark conditions. Cones consist of the photopsin pigments I, II, and III. Each type of photopsin pigment detects a different wavelength that corresponds to the colours blue, green, or red, respectively. Iodine and opsin are also constituents of cone cells and support their visual functions. Moreover, cone cells are concentrated in the fovea, the central region of the retina, which is key for central vision.

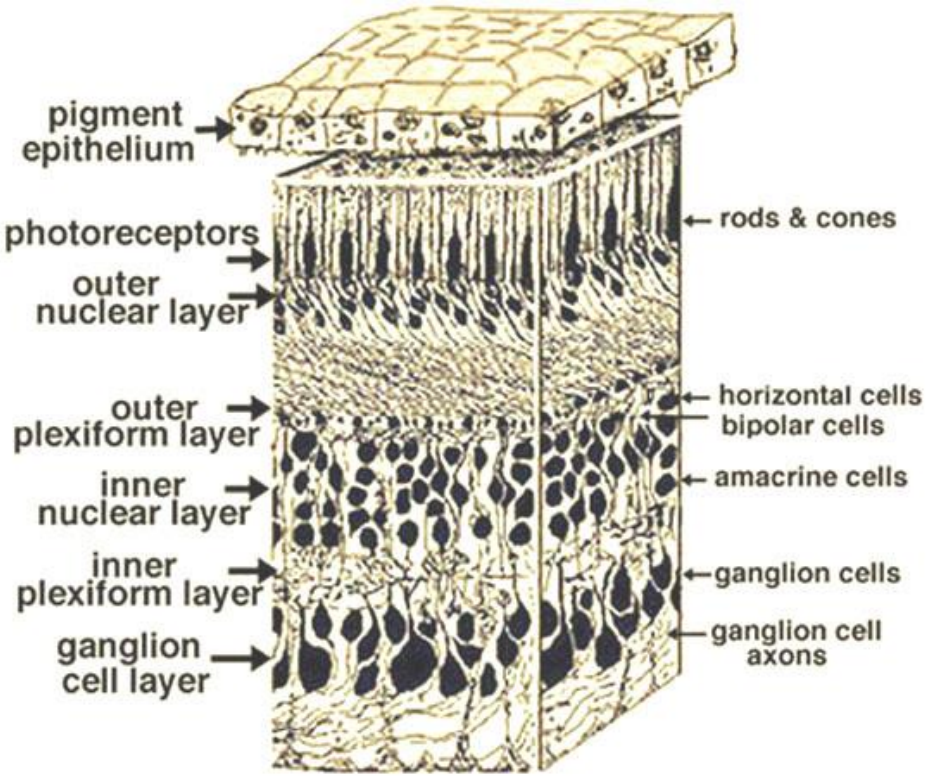

Figure 1-1. Layers of the retina and their components (Kolb et al., 2007).

When light enters the retina, rod cells and cone cells found in the photoreceptors are responsible for scotopic and photopic visual processing, respectively. These photoreceptor cells are interacting with the horizontal cells and bipolar cells through synaptic connections in the OPL (Kolb et al., 2007). Amacrine cells, which are essential in modulating the activity between bipolar cells and ganglion cells through neurotransmitters, are located in the IPL. Finally, the GCL contains ganglion cells, which are crucial to propagate visual activity to the brain through the optic nerve.

The Visual Cycle

Phototransduction begins when photons of light enter the rod and isomerize 11-*cis* retinal molecules into all-*trans*-retinal (Fu, 2007). This transduction reaction cleaves and releases the

protein opsin, which triggers a cascade of electrical and chemical events that are sent to the ganglion cells and eventually reach the brain. This reaction occurs millions of times every day. Therefore, constant regeneration of the retina is necessary to meet the demand needed to support the visual cycle. Indeed, following light absorption, all-*trans*-retinal is reduced to all-*trans*-retinol and shuttled to the cells in the RPE. Following multiple conversions, all-*trans*-retinol returns to its 11-*cis*-retinal form, which then enters the photoreceptor cells and becomes available once again to support the visual cycle.

The pathways involved in supporting the visual cycle are constantly in flux and require a substantial number of essential metabolites. Due to this never-ending need, the RPE, in conjunction with the choroid, also functions to transport essential nutrients (Strauss, 2007). For example, retinal is taken up to supply opsin, which supports the visual cycle. Omega-3 fatty acids, such as docosahexaenoic acid (*DHA, C22:6n-3),* are important components of the photoreceptor membrane, and glucose is necessary for energy metabolism in the retina. Consequently, the renewal of essential nutrients such as these could generate high concentrations of reactive oxygen species (ROS), resulting in retinal cell damage. The RPE functions to protect the retina from severe damage by maintaining its integrity through immune modulation. The retina contains high levels of macular pigments, which function as antioxidants to modulate the immune system.

Measuring Retina Function with Full-Field Electroretinography

When the retina is exposed to light, the photoreceptors and other retinal cells elicit electrical potentials, known as electroretinogram (ERG) responses (McAnany et al., 2022). These electrical signals will leave the retina and enter the cornea, where they can be detected. Full-field electroretinograms (ffERGs) will flash specific lights into the eye to trigger ERG

responses. Then the sensors attached to the ERG device will detect these electrical responses and compare the results to reference databases to assess retinal function.

The ffERG recording appears as a waveform on a chart, with the X-axis representing the implicit time (in milliseconds) and the Y-axis representing the response amplitude (in microvolts) (McAnany et al., 2022). The first component is the negative a-wave, which reflects the outer retinal function of the rod and cone cells. Second is the positive b-wave, which represents the electrical response from inner retinal Muller and bipolar cells (**Figure 1-2**). This response indicates the integrity of the phototransduction pathway. Additionally, oscillatory potentials (OP) are wavelengths situated on top of b-waves and reflect the electrical activity of amacrine cells. In light-adapted conditions, the transmitted signal flows from the photoreceptors to the inner retina, and in dark-adapted conditions, the signal flows to the rods. The final major component is the photopic negative response, which represents the electrical response from the bipolar cells to the ganglion retinal cells. The peak latencies and the OP of these waves can provide insight into any dysfunction occurring in those cells. Considering the significant impact vision loss has on quality of life, using RETeval to assess retinal function in at-risk populations could be a priority.

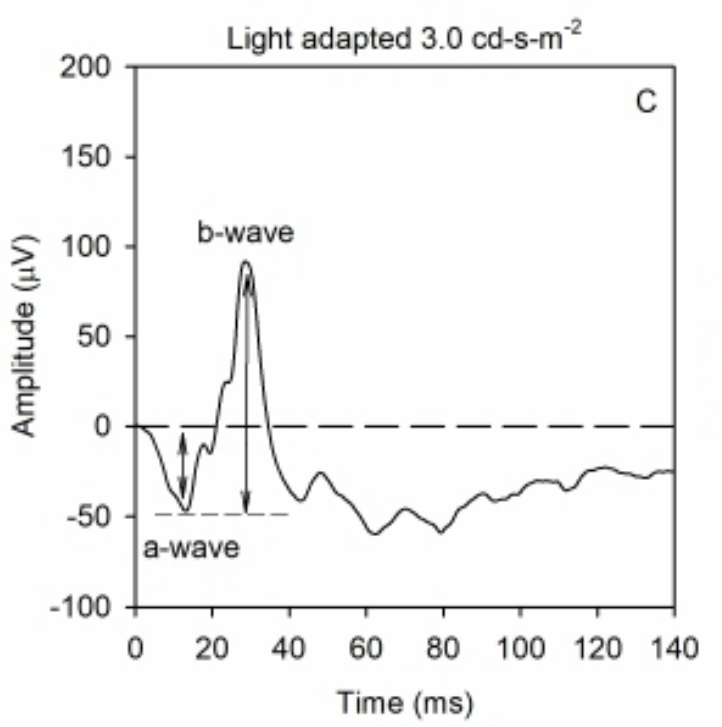

Figure 1-2. ERG recording results (McAnany, J., 2023).

Diabetes

Diabetes is a major global health issue, nearing epidemic proportions. In 2017, approximately 425 million people were diagnosed with diabetes worldwide, and researchers forecast that this number will increase by 200 million during the next 20 years (Standl et al., 2019). Type 2 diabetes (T2DM) stands as the primary factor in diabetes prevalence because it represents more than 90% of total cases. The pathophysiology of T2DM begins with pancreatic insulin production defects and insulin resistance in insulin-sensitive tissues (Galicia-Garcia et al., 2020). Together, these mechanisms will increase blood glucose concentration, and a diagnosis of T2DM is confirmed once those concentrations are ≥ 7 mmol/L (Standl et al., 2019). If left untreated, increased blood glucose concentrations can damage the vasculature and lead to severe complications.

Diabetic Retinopathy

Diabetic retinopathy is a significant complication of diabetes, resulting from prolonged elevated blood glucose levels in the retinal vasculature (Kusuhara et al., 2018). The blood vessels in the retina are extremely sensitive and can deteriorate if damaged. Chronic elevation of blood glucose levels can damage these vessels over time, leading to retinopathy. The loss of vision creates significant challenges to independence and personal ability, which severely affects quality of life.

Prevalence

The increase in diabetes worldwide leads to a rapid growth in diabetic retinopathy cases. Research indicates that diabetic retinopathy affects 131 million people worldwide, while vision-threatening cases affect 31 million (23.7%) (Teo et al., 2021). They also predict that the number will increase to 205 million by 2045. The vast majority (99%) of individuals with type 1 diabetes (T1DM) will develop some retinopathy during their lifetime, as will more than 60% of individuals with T2DM, representing the leading cause of blindness in developed countries (Klein et al., 2008; Teo et al., 2021). The loss of vision impacts all facets of life, including independence, health, and overall quality of life.

Risk Factors

The most significant risk factor for diabetic retinopathy is duration of diabetes (Lin et al., 2020). Elevated blood glucose levels can damage the blood vessels of the retina, leading to leakage, blockages, or other changes in the vasculature. The longer an individual has diabetes, the greater the risk of developing these damages. Other important risk factors include hyperglycemia and hypertension. As previously mentioned, diabetic retinopathy is a disease of the blood vessels; therefore, any conditions that directly target the integrity of blood vessels will likely increase the risk of diabetic retinopathy. The Hoorn study (2003) demonstrated that

diabetic patients with hypertension face double the risk of developing diabetic retinopathy during the following ten years because high blood pressure damages the inner endothelial cells of blood vessels (van Leiden et al., 2003). As the endothelium becomes dysfunctional, nitric oxide production decreases, resulting in vasoconstriction and reduced blood flow (Lin et al., 2020). The underlying mechanistic role of hyperglycemia in the development of diabetic retinopathy is not fully understood. However, one accepted theory is that elevated blood glucose levels cause biochemical changes within the retinal vasculature, leading to structural abnormalities (Lin et al., 2020). Another mechanism is through increasing vascular wall permeability. As leakage occurs, the retina may begin to swell, leading to macular edema.

Table 1-1. Defining features of the developmental stages of diabetic retinopathy

DR Severity	Defining features
No DR	No microvascular abnormalities
Mild NPDR	Microaneurysms only
Moderate NPDR	Microaneurysms and other signs (dot and blot hemorrhages, hard exudates, cotton wool spots)
Severe NPDR	Intraretinal hemorrhages (>20 in each of 4 quadrants), definite venous beading (in at least 2 quadrants), or apparent IRMA (in at least 1 quadrant)
PDR	Neovascularization of optic disc or elsewhere, preretinal hemorrhage, or vitreous hemorrhages

Diabetic retinopathy: DR; Non-proliferative diabetic retinopathy: NPDR; Proliferative diabetic retinopathy: PDR (Kusuhara et al., 2018).

Developmental Stages of Diabetic Retinopathy

Mild and Moderate Nonproliferative Diabetic Retinopathy

The earliest stages of diabetic retinopathy are characterized by the formation of microaneurysms in the retinal vasculature (Lin et al., 2020) (Table 1-1). Microaneurysms are small outpouchings that form in the blood vessels when the endothelium, the innermost layer of the blood vessel, is damaged and weakened. Despite the presence of microaneurysms, individuals do not typically experience noticeable symptoms at this stage. As the microaneurysms continue to swell, leakage of blood and fluid into the retina can occur, resulting in the formation of hard exudates. In addition to the hemorrhages, blood flow will also be compromised, restricting oxygen and nutrient delivery to the retinal cells, which can result in cotton wool spots. Moderate non-proliferative diabetic retinopathy will advance to severe when the number of intraretinal hemorrhages or bleeding increases.

Severe Non-proliferative Diabetic Retinopathy

Severe NPDR is determined if any of the following three characteristics are present during an optical coherence tomography exam (Lin et al., 2020) (Table 1-1). More than 20 intraretinal hemorrhages or microaneurysms are present in each of the four retinal quadrants. If venous beading is present in at least two quadrants. Suppose any intraretinal microvascular abnormalities from excessive leakage are present. At this stage, chronic damage to the retinal vasculature has restricted blood flow to specific areas, resulting in the neovascularization of thin and fragile blood vessels. Additionally, blood and fluid leakage cause swelling and thickening of the retinal tissue, which contributes to macular edema. At this stage, blurred vision, dark spots, and some loss of vision are likely and may be permanent.

Proliferative Diabetic Retinopathy

The new abnormal blood vessels in this stage can be damaged and bleed into the vitreous, which blocks light from reaching the retina (Lin et al., 2020). The blood vessels at this stage can develop scar tissue that creates pressure on the retina, leading to retinal detachment. Blurry vision, distorted vision, and loss of vision are common at this stage of diabetic retinopathy.

Current Treatments for Diabetic Retinopathy

The vision loss from diabetic retinopathy remains irreversible. However, a comprehensive approach exists to stop the disease from advancing to more severe stages. The management of mild and moderate diabetic retinopathy depends on maintaining controlled blood glucose levels and hypertension. The treatment options for severe and proliferative diabetic retinopathy patients include photocoagulation therapy, anti-VEGF treatment, and vitrectomy. The gold standard treatment for severe NPDR and PDR is currently photocoagulation therapy using argon lasers (Moutray et al., 2018). It involves using a laser to seal or destroy abnormal blood vessels in the retina, thereby reducing leakage and swelling. A recent review compared modern laser techniques to standard argon lasers, and currently, there is limited evidence to support their use (Moutray et al., 2018). VEGF is a key protein that stimulates the development of new, weakened blood vessels in the retina (Arrigo et al., 2022). Anti-VEGF medications are injected into the vitreous gel with a needle, and by preventing neovascularization from occurring, they also prevent leakage from those vessels. Vitrectomy surgery will only be recommended for advanced cases after trying every other possible treatment (Naresh Shah et al., 2020). The surgery involves making small incisions in the white part of the eye and surgically removing the vitreous gel, containing blood and scar tissue, from the retina surface.

Nutrients Involved in Retina Function

Docosahexaenoic acid

Docosahexaenoic acid (DHA, C22:6n-3) is a beneficial omega-3 polyunsaturated fatty acid (PUFA) with 22 carbons. Omega-3 fatty acids, consisting of alpha-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), and DHA, serve essential functions for both cardiovascular health and diabetes management (Lafuente et al., 2021). The retina specifically depends on DHA for its proper functioning since this fatty acid makes up 60% of photoreceptor membranes (Agbaga et al., 2018). The human body lacks sufficient enzymes to synthesize this nutrient effectively; therefore, adequate dietary intake of DHA is necessary. Fish, including salmon, tuna, cod , and mackerel, serve as primary sources of DHA , while ALA exists in plant-based foods such as flaxseed, chia seed, algae , and spirulina (Cholewski et al., 2018).

Absorption and Metabolism

DHA exists as triacylglycerol (TAG) in food. After consumption, TAGs undergo digestion in the stomach through gastric lipases and then move to the small intestine (Punia et al., 2019). The presence of TAGs activates cholecystokinin secretion, which leads to bile production for emulsifying the long-chain fatty acids. TAGs become fatty acids (FAs) and monoacylglycerols after pancreatic lipase breaks them down. Long-chain fatty acids, together with 2-monoglycerides and other lipid compounds, need to form micelles before they can pass through enterocytes. After circulating through the body, it reaches the liver, where it is metabolized.

Alternatively, DHA can be synthesized from ALA (Punia et al., 2019). This metabolic pathway begins with stearidonic acid (SDA, C18:4n-3), which is converted into EPA, DPA, and

ultimately DHA. The seven-step conversion requires two desaturase enzymes (delta-5- and delta-6-desaturases), three elongase enzymes, and concludes with peroxisomal chain shortening through beta-oxidation. This conversion pathway is known to be ineffective in producing DHA, with only 1% of ALA being converted to DHA in humans (Domenichiello et al., 2015), a fact that underscores DHA's essential or semi-essential nature in the literature.

DHA in the Retina

The retina depends on DHA to maintain its visual functions and achieve optimal retinal health. This dependency is because DHA accounts for 50-70% of rod photoreceptors (Agbaga et al., 2018). The photoreceptor membrane structure and functionality reach their optimal state because DHA maintains membrane fluidity. Additionally, DHA enables rhodopsin to undergo conformational changes, which initiate chemical reactions in the phototransduction pathway (Lafuente et al., 2021). Finally, DHA also supports retinal health by reducing oxidative stress and inflammation, which are key factors in retinal diseases such as diabetic retinopathy (Lafuente et al., 2021). The following section discusses the effectiveness of DHA trials in humans and animals in retinal diseases.

Human Trials

DHA Supplemental Trial

The role of DHA supplementation in preventing retinal diseases remains unclear in humans. Over a 2-year period, García et al. (2022) investigated the effect of a highly concentrated DHA supplement (1,050 mg/day) on slowing the progression of any stage of NPDR. Individuals with NPDR (n = 170) were randomly assigned to either a DHA supplement or olive oil as a control group, with visual function and blood tests conducted every 6 months. At

the end of the study, no significant difference was found between the trial groups in preventing the progression of NPDR or improving visual acuity.

The combination of DHA and macular pigment supplementation appears promising for preventing the progression of diabetic retinopathy. González-Herrero et al. (2018) investigated the effect of a DHA (1,050mg/day) and carotenoid (9 mg of lutein and 1 mg of zeaxanthin) supplement on retinal function in individuals with NPDR. Individuals with T2DM and NPDR (n = 31) were randomly assigned to either the supplement or control group, and assessments were conducted at baseline, day 45, and at the end of the intervention (day 90). Microperimetry was used to assess macular function, and visual acuity and macular thickness were also evaluated. At the end of the study, the DHA and carotenoid-supplemented group demonstrated a significant improvement in macular sensitivity and macular integrity (macular function) compared to the control group. The DHA supplementation group also exhibited a significant increase in plasma total antioxidant capacity values, which may explain the protective role of the supplements in the retina. Overall, no differences in visual acuity and macular thickness were observed. Although the role of DHA supplementation in treating diabetic retinopathy remains unclear, there is potential for the combined supplementation of DHA and macular pigments, such as lutein, to prevent the disease.

DHA Intake Trials

Population-based studies have shown that omega-3 PUFAs in diets create a lasting protective effect against diabetic retinopathy. A cross-sectional study involving 379 participants with diabetes was conducted to investigate the relationship between PUFA consumption, blood glucose control, and the development of diabetic retinopathy (Sasaki et al., 2015). Dietary intake was measured using semi-quantitative food frequency questionnaires (FFQ), and diabetic

retinopathy was graded based on fundus photographs. Overall, no significant association was found between the consumption of PUFAs and the risk of diabetic retinopathy. Interestingly, the research revealed that better-controlled diabetic patients who consumed higher amounts of PUFAs demonstrated reduced diabetic retinopathy risk after adjusting for other influencing variables. These findings align with Sala-Vila et al (2016), who discovered that omega-3 PUFA consumption reduced the incidence of severe diabetic retinopathy that could lead to blindness. This prospective study divided 3482 participants with T2DM into two groups: those who consumed more than 500 mg/day of omega-3 PUFAs and those who did not. Dietary intake was measured using a semi-quantitative FFQ every year for 6 years. The 6-year follow-up data indicated that individuals who consumed more than 500 mg/day of omega-3 PUFA at baseline had a 46% reduced risk of developing sight-threatening diabetic retinopathy after adjusting for confounding factors. However, these studies did not measure retinal function with ERG.

A more recent cross-sectional study also found a positive association between the consumption of DHA and the presence and severity of diabetic retinopathy in individuals with type 2 diabetes mellitus (T2DM) (Weir et al., 2023). Participants (n = 1,356) were selected with varying degrees of diabetic retinopathy, and blood levels of DHA and EPA were used to assess their dietary intake. Interestingly, only plasma DHA had an inverse relationship with the presence and severity of diabetic retinopathy. They found that those in the 75th percentile and higher of dietary DHA intake were 17% less likely to develop diabetic retinopathy compared to individuals in the first 25th percentile. Additionally, those in the 75th percentile and higher group also had a 38% reduction in the severity of diabetic retinopathy compared to those in the first 25th percentile.

Animal Trials

Clinical research exploring the role of DHA and diabetic retinopathy is limited, but multiple animal models of type 1 and T2DM have been used to explore this relationship. Overall, the findings in animal models are consistent in demonstrating the beneficial effect of DHA in preventing the retinal damage associated with diabetic retinopathy. Sapieha et al (2012) investigated the effect of omega-3 PUFA on the retinal function of T2DM mice. The trial group consumed a diet enriched with omega-3 polyunsaturated fatty acids (2% DHA and EPA) versus omega-6 polyunsaturated fatty acids (2% arachidonic acid, AA, C20:4n-6). After the 22-week intervention, the retinal function of the omega-3 PUFA group was comparable to that of the control non-diabetic mice, demonstrating the protective effect of omega-3 PUFA on retinal health. Specifically, the omega-3 PUFA group showed improvements in both scotopic a-wave and b-wave responses, including OP, indicating reduced retinal cell degradation. It is worth noting that animals in the omega-3 PUFA group also had lower blood glucose levels, which likely contributed to some of the benefits observed in the retina. A similar study was conducted by Kowluru et al. (2014), which investigated retinal function in diabetic rats consuming an omega-3 PUFA and carotenoid-rich supplement. Retina function was also assessed using an ERG. After 4 months, the diabetic mice exhibited a significant reduction in a-wave and b-wave amplitudes, indicating retinal cell deterioration. In accordance with the research by Sapieha et al (2022), the omega-3 PUFA and carotenoid supplement attenuated the reduction in amplitudes of both a-wave and b-waves, demonstrating the protective role of both omega-3 PUFA and carotenoids in retinal cell health. Additionally, Kowluru et al. (2014) also found that the levels of reactive oxygen species (ROS) were significantly lower in the retinas of diabetic mice consuming the supplement, with a higher total antioxidant capacity, a general marker of

antioxidant potential. Together, these results demonstrate that the supplement significantly decreased retinal oxidative stress in the diabetic group compared to the non-supplemented group. Animal studies consistently demonstrate the beneficial effects of DHA on retinal cell integrity, although further clinical trials are needed to confirm these results.

Lutein

Lutein is one of the 700 compounds found in the carotenoid family (Mitra et al., 2021). The antioxidant properties of carotenoids have been shown to decrease inflammation and oxidative stress. Research has established that higher carotenoid consumption is associated with a decreased risk of cardiovascular disease (Wood et al., 2014), reduced cancer risk (Aune et al., 2018), and a lower risk of osteoporosis (Kan et al., 2021). Carotenoids consist of two main structural classes, which include carotenes and xanthophylls (Mitra et al., 2021). The former consists of hydrocarbons, such as β-carotene and lycopene, and the latter contains oxygen atoms in the form of hydroxy or carbonyl groups. Lutein, a xanthophyll, has a unique structure with a hydroxyl group at both ends, which is responsible for its enhanced reactivity to singlet oxygen (**Figure 1-3**). Lutein's chemical formula consists of 40 carbon atoms and six double carbon-carbon bonds. Since humans cannot produce lutein, it is essential to consume dietary or supplemental forms of lutein. Lutein is most commonly found in green, leafy vegetables (such as kale, spinach, and romaine lettuce), corn, bell peppers, pumpkin, and certain squashes (Abdel-Aal et al., 2013).

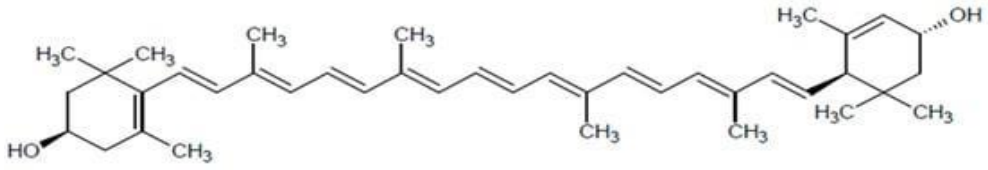

Figure 1-3. Structure of lutein (Buscemi et al., 2018).

Absorption and Metabolism

The digestive enzymes quickly separate dietary lutein from its food matrix after consumption (Buscemi et al., 2018). The small intestine breaks down lutein through bile salts and lipids, which creates mixed micelles that enable its absorption. The amount of lipid intake during digestion determines the rate at which lutein passes through the intestinal epithelium. Following absorption, the chylomicrons are secreted into the lymphatic system and transported to the liver, where they are broken down into their individual components. Lutein enters the bloodstream through lipoproteins to reach various tissues, including the retina. The retina transports lutein through direct delivery to the RPE cells before processing occurs for photoreceptor cell distribution in the macula. The retina uses selective transport mechanisms to guarantee that optimal lutein concentrations are maintained.

Lutein in the Retina

Lutein, along with zeaxanthin and meso-zeaxanthin, helps preserve retinal health in the macula region, where they are highly concentrated (Tuj Johra et al., 2020). These three carotenoids function as antioxidants and blue light filters. As previously explained, the retina is highly susceptible to oxidative stress because of its constant exposure to light, a high PUFA environment, and high metabolic demand. Additionally, diabetic retinopathy increases the oxidative stress placed on the retina (Lima et al., 2016). Carotenoids function as antioxidants to neutralize free radicals, which reduces oxidative damage to the photoreceptor cells and other retinal cells. Ultimately, oxidative damage is a fundamental factor in the pathogenesis of retinal

diseases, particularly diabetic retinopathy. The protective role of macular pigments in such retinal inflammatory diseases is currently being investigated

Human Trials

Supplemental Form of Lutein

Hu et al (2011) explored the effect of lutein and zeaxanthin supplementation on visual function and serum lutein and zeaxanthin levels in individuals with NPDR. A functional acuity contrast test was used to assess visual function, as it is a good indicator of retinal health in individuals with diabetes. Following the three-month supplementation (lutein (6mg) and zeaxanthin (0.5mg)), contrast sensitivity significantly improved in the NPDR lutein and zeaxanthin treatment group (n=30) compared to the NPDR control group (n=30). The researchers hypothesize that in addition to the supplementation's antioxidant effect, lutein and zeaxanthin also improve visual acuity by signal transduction through the visual system. Overall, these results further establish the potential of lutein and zeaxanthin supplements to improve vision in individuals with diabetes.

Lutein Intake Trials

Population-based studies have demonstrated a consistent positive relationship between dietary intake of lutein and a reduced risk of diabetic retinopathy. Brazionis et al (2009) performed a cross-sectional study to determine if a relationship existed between dietary carotenoid intake and the presence of the disease. Plasma carotenoid levels of individuals with T2DM (n=111) were measured. After adjusting for confounding variables, the researchers found that higher plasma concentration of lycopene, lutein, and zeaxanthin was correlated with

significantly lower rates of diabetic retinopathy. These results suggest a possible relationship between lutein and zeaxanthin intake and the diminishing risk of diabetic retinopathy

Animal Trials

Multiple animal studies have investigated the role of lutein supplementation on oxidative stress markers and retinal health. Overall, we investigated retina function and oxidative stress markers in alloxan-treated (200 mg/kg body weight) T1DM mice that were fed lutein (0.2 mg/kg body weight). Diabetic and healthy control mice were then subdivided into two groups: one receiving a chow diet and the other receiving a chow diet supplemented with lutein. Following the 2-week intervention, the ERG b-wave amplitude of the diabetic mice consuming lutein was significantly higher than that of the diabetic mice without lutein. Similarly, the oxidative stress marker malondialdehyde (MDA) was restored to a control level in diabetic mice consuming lutein. These results suggest that a lutein treatment can alleviate oxidative stress in the retina and prevent retinal cell deterioration in diabetic conditions. These results are consistent with those of Deshpande et al. (2016), who examined retinal damage and oxidative stress markers in streptozotocin-induced T1DM mice that consumed a lutein and zeaxanthin supplement. Following the 12-week intervention, diabetic mice fed the lutein and zeaxanthin treatment exhibited reduced oscillatory potentials compared to the diabetic group without supplementation. The researchers also found that the diabetic mice without supplementation had lower levels of manganese superoxide dismutase and glutathione, two crucial ROS scavenging enzymes. Although non-diabetic blood glucose levels were not restored by the lutein and zeaxanthin treatment, its effect on ERG b-wave amplitude and antioxidant enzymes demonstrates favourable changes in the diabetic retina.

Similar findings were also observed by Wang et al. (2020), who investigated the role of long-term lutein supplementation (4.2 mg/kg/day) on retinal function in T2DM mice. After 9 months, the ERG showed that diabetic mice had lower scotopic a-wave and b-wave amplitudes, indicating deterioration in the inner retinal cells. Interestingly, lutein supplementation attenuated the decrease in scotopic a-wave and b-wave amplitudes, demonstrating the protective effect of lutein supplementation, which may protect retinal cells from diabetes-induced damage. There is ample evidence from animal studies demonstrating lutein's ability to prevent retinal cell damage that occurs in diabetic retinopathy. Whether similar findings can be found in clinical trials remains to be discovered.

Chapter Flows

This thesis is written in manuscript style and consists of four chapters, representing original work completed in fulfillment of my MSc degree requirements.

Chapter 1 provides an introduction to the topic and a literature review, which critically assesses the current state of knowledge surrounding the retina, diabetic retinopathy, and the role of nutrients in retinal health

Chapter 2 presents data on the current status of retinal health, macular pigment levels, and metabolic parameters in individuals with type 2 diabetes mellitus (T2DM) compared to a non-diabetic group.

The overarching hypothesis for this chapter is that individuals with T2DM have poorer retinal health, lower macular pigment density, and less favorable metabolic parameters than non-DM individuals.

Chapter 3 investigates the effects of consuming DHA- and lutein-enriched eggs on retinal health, macular pigment density, and metabolic parameters in individuals with T2DM compared to non-diabetic controls.

The hypothesis is that consumption of lutein- and DHA-enriched eggs will improve retinal function in individuals with T2DM without negatively impacting their plasma metabolic profile.

Chapter 4 presents a final summary and conclusion of the study

Two manuscripts are currently in preparation:

1. Candas J et al. (2025) Exploring Objective and Subjective Indicators of Vision Health in T2DM Without Retinopathy Compared to Non-Diabetic Individuals (*Nutritional Neurosciences*)

2. Candas J et al. (2025) Effects of DHA and lutein enriched eggs on retina health and metabolic parameters in individuals with type 2 diabetes (*British Journal of Nutrition*)

Chapter 2. Exploring Objective and Subjective Indicators of Vision Health in T2DM Without Retinopathy Compared to Non-Diabetic Individuals

Abstract

Vision impairment is a significant concern among individuals with diabetes as a consequence of diabetic retinopathy. However, retinopathy is often not noticeable in the early stages, especially in the slow progression of type 2 diabetes mellitus (T2DM) and later can be confounded by vision decline related to aging. Therefore, this study examined the following: i) retinal health and macular pigment levels in individuals diagnosed with T2DM without having retinopathy. ii) the consistency between individuals' subjective perceptions of their vision health and objective measurements of retinal function. iii) The association between carotenoid status and macular pigment density. Thirty-four individuals with T2DM (44% f, 56% m; 61 ± 12 yrs) and 30 individuals with normal glucose metabolism (NGM) (67% f, 33% m; 55 ± 13 yrs) were recruited. The perception of vision health and diet quality was assessed using the validated 'Nutrition and My Retina' questionnaire. Retina function was assessed by electroretinogram and macular pigment optical density measurements by heterochromatic flicker photometry. Other objective assessments included skin-based carotenoid scores, advanced glycation end products (AGE), body weight and composition, and plasma metabolic parameters. Three-day dietary records were used for dietary assessment. As expected, the T2DM group had significantly higher plasma glucose concentrations and AGE scores compared to the NGM group ($p<0.01$). Both T2DM and NGM groups primarily did not notice any vision impairment; however, ERG for the T2DM group showed significantly lower retina photoreceptor cell implicit time responses ($p<0.01$), indicating a slower response of the retina cells compared to the NGM group. The T2DM group had significantly lower skin-based carotenoid scores compared to the NGM group ($p<0.05$); however, there was no correlation between skin carotenoid scores and MPOD. Both groups reported a low belief in food influencing eye health. The T2DM group consumed more than the recommended amounts of dietary fat, saturated fat, and cholesterol. Overall, this study indicated there are discrepancies between perceived and actual retina function and eye-beneficial dietary practices among people with T2DM, underscoring the importance of objective assessments in early detection of retinal abnormalities. Since the retina is enriched with specific nutrients such as lutein and DHA, including these eye-beneficial nutrients in the diet may improve retina health in people with diabetes, especially before the onset of clinically detectable complications.

Keywords: Electroretinogram, Macular Pigment Optical Density, Type 2 Diabetes,

Introduction

Diabetes is a major global health issue, nearing epidemic proportions (Public Health Agency of Canada, 2023). Type 2 diabetes (T2DM) is the most common form of diabetes, accounting for more than 90% of total cases (Galicia-Garcia et al., 2020). Elevated blood glucose levels, characteristic of T2DM, can damage blood vessel walls over time, leading to more severe complications. Diabetes contributes to 40% of strokes, 30% of heart attacks, 50% of kidney failure requiring dialysis, and is the leading cause of blindness both in Canada and worldwide (Public Health Agency of Canada, 2023).

Diabetic retinopathy is a major complication of T2DM caused by prolonged elevated blood glucose levels in the retinal vasculature (Kusuhara et al., 2018). Approximately 60% of those with T2DM will have some degree of retinopathy during their lifetime. Diabetic retinopathy develops gradually and is often only noticed in the later, more severe stages, when symptoms like blurriness and vision loss become apparent. Once diabetes has caused damage to the retina, vision loss can be permanent, significantly impacting quality of life. Therefore, it is imperative to detect early.

Recent advances in diabetic retinopathy research have demonstrated that abnormal changes in retinal cell function precede the onset of the condition's visual symptoms (McAnany et al., 2021). Electroretinograms (ERG) provide an objective measurement of retinal function by assessing the reaction time and strength of retinal cell responses (McAnany et al., 2021). While research shows that individuals with T2DM have abnormal retinal cells compared to those without diabetes, the specific ERG patterns most affected by diabetes remain unclear. Notably, many individuals with T2DM are unaware of any vision difficulties, despite these early functional changes.

Macular pigments concentrated in the central retina, such as lutein and zeaxanthin, found in fruits, vegetables, and egg yolks, protect the retina by neutralizing harmful free radicals, thus reducing oxidative damage to the retinal cells (Tuj-Johra et al., 2020). Macular pigments have been shown to have protective effects against certain eye diseases, such as age-related macular degeneration (Bernstein et al., 2010); however, their effect in diabetic conditions is not well understood.

Therefore, the purpose of this study is to examine retinal health and macular pigment levels in individuals diagnosed with T2DM who do not have retinopathy. In addition, the study evaluates the consistency between individuals' subjective perceptions of their vision health and objective measurements of retinal function. Carotenoid status is also assessed to explore its association with macular pigment density. Ultimately, this study will provide a comprehensive understanding of the factors influencing vision health in individuals with T2DM, using a non-diabetic group as a comparison.

Experimental Design and Measurement

Participants and Study Design

Participants

A total of 64 participants, including 34 individuals with T2DM (n=19 men and 15 women) and 30 individuals with normal glucose metabolism (NGM) (n=10 men and 20 women), were recruited via advertisements through various media from Winnipeg, Manitoba, Canada, and the surrounding areas between July 2022 and February 2024.

Inclusion criteria for the T2DM group were male or female over the age of 18; a previous diagnosis of T2DM, thus currently taking oral hypoglycemic medications. Inclusion criteria for the NGM group included male or female over the age of 18; not currently diagnosed with pre-

diabetes or T2DM; and not taking oral hypoglycemic medications. Both T2DM and NGM groups have not donated blood in the past two months and are willing not to donate for the duration of this trial; has not in the last month participated in another dietary intervention trial and are willing not to start one for the duration of this study; and willingness to comply with the protocol requirements and provide informed consent.

Exclusion criteria for the T2DM and NGM groups were previous diagnosis with diabetic retinopathy; previous diagnosis of eye disease, mental cognitive disease, type 1 diabetes; taking insulin; diagnosed with cancer in the last 5 years; currently (within the past 3 months) diagnosed with anemia; currently pregnant or breastfeeding; taking a multivitamin, DHA and/or mineral supplements that contain vitamin A, lutein, zeaxanthin, omega-3, DHA, zinc or beta-carotene within 30 days prior to study enrollment; a bacterial, viral, or fungal infection within 30 days prior to study enrolment; any medical conditions or surgical interventions within 3 months of the first study visit; inability to take prescribed medications without food; inability to fast overnight; and sensitivity to flashing lights.

Study Design

The present study was conducted at the I.H. Asper Clinical Research Institute at St. Boniface Hospital in Winnipeg, Manitoba, Canada, from January 2023 to January 2025. his study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Approval was granted by the University of Manitoba Research Ethics Board (HS23602, B2020:007) and the St. Boniface Hospital Research Ethics Committee (RRC/2020/1964). All participants provided written informed consent consistent with guidelines for the protection of human research participants. The study protocol was registered on ClinicalTrials.gov (NCT04496817).

This trial employed a single-site, cross-sectional, observational design, where participants were assessed at two visits, 3 weeks apart, under free-living conditions. Participants were scheduled to arrive following a 12-hour overnight fast. A Study Coordinator conducted visits for the following sampling and measurements: fasted blood samples, retina and macular health, metabolic parameters, body composition, blood pressure, skin-based carotenoid status, a three-day food record, and a self-reported questionnaire on social-demographics, health, and visual function.

Study Procedures and Measurements

Retina and Macular Health

Electroretinogram

Changes in retinal function were ascertained by measuring parameters related to the ffERG waveform in response to flashing light stimuli: time of response to light (implicit time) and the strength of the response (maximum amplitude) (McAnany et al., 2022). The full-field electroretinography (ffERG) was used to assess retinal cell function, including rods and cones using the *RETeval*® (LKC Technologies, Gaithersburg, MD, USA). The ffERG responses were recorded using disposable sensor strip electrodes placed 2 mm inferior to each lower eyelid margin and aligned with the participants' non-dilated pupils, following the manufacturer's guidelines. Light-adapted, photopic response was performed using an 85 Td·s stimulus at 28.3 Hz flicker within a small 60 mm Ganzfeld dome. Both eyes were tested sequentially, starting with the right eye while the contralateral eye remained covered. Measurements were performed in duplicate. Participants then underwent a 10-minute dark-adaptation period in a light-free room.

During the dark-adapted testing, scotopic ERG testing of rod cells was performed under a dim red-light illumination with the *RETeval*® device delivering intermittent flashes with an intensity of 85 Td·s, at 0.1 Hz flicker. Eye position stability was instructed by encouraging fixation on a red central dot inside the stimulator, with fixation monitored by viewing the pupil through the *RETeval*® device. Both eyes were tested sequentially, and measurements were performed in duplicate.

The ERG recording produces a biphasic waveform plotted on a chart, with the X-axis representing the response time (implicit time) in milliseconds and the Y-axis representing response strength (amplitude) in microvolts (McAnany et al., 2022). The waveform consists of three main components: the a-wave, the b-wave, and the oscillatory potentials (OPs). The a-wave reflects outer retinal function, primarily from rod and cone photoreceptors. The b-wave corresponds to activity in the inner retinal Müller and bipolar cells. Finally, the OPs represent the electrical activity of amacrine cells. Under light-adapted conditions, the signal flow primarily involves cone photoreceptors transmitting to the inner retina, while under dark-adapted conditions, the signal flow is dominated by rod photoreceptors.

A total of 62 participants completed the photopic ERG assessment, and 56 participants completed the scotopic ERG assessment. The scotopic assessment was considerably longer, making it challenging for participants to keep their eyes open for long enough to obtain accurate readings

Macular pigment optical density

Macular pigments were quantified in terms of macular pigment optical density (MPOD), which was measured using the heterochromatic flicker photometer Quantify MPS II system (Premier Eyecare, Knoxville, TN, USA, ZeaVision LLC, Chesterfield, MO, USA. While sitting

down, the participant was asked to look into the machine with one eye and fixate a central 1 ° target. Once this central target starts to flicker, they were instructed to press a button and continue looking for the next flicker. The detailed protocol is as follows: The target in the system will alternate between a green (530nm) and blue (465nm) LED light with luminance up to 200 cd/m2. Throughout the assessment, these lights start to flicker at a rate of 60 Hz per second gradually slowing to 6 Hz per second. The blue light is absorbed by the macular pigments, while the green light is not. Initially, the target appears static, but as the flickering rate between the blue and green light slows, a flicker appears, and the participant clicks the button. Measurements are obtained from the blue and green light ratios at the time the participant clicked the button.
For peripheral vision, participants were asked to fixate on a 2° red spot located 8° horizontally from the central target. The same procedure stated above was repeated, and participants were asked to press the button when they observed a flicker in the central target using their peripheral vision. Once finished, the MPOD calculation is based on the endpoint when each participant pressed the response button and the corresponding blue and green light ratio. The MPOD score ranges from 0 to 1, with scores of 0-0.21 being considered very inadequate, 0.21-0.5 being inadequate, 0.5-0.7 being adequate, and 0.7-1 being excellent (Van Der Veen et al., 2009). Measurements were repeated twice, and for each eye test, the contralateral eye was closed or covered.

Plasma Metabolites Parameters

Plasma Biochemistry

Fasted plasma glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, and hs-C-reactive protein (hs-CRP) were determined using the automated Roche Diagnostics Cobas c 111 Analyzer (F. Hoffman-La Roche Ltd., Basel, Switzerland).

Plasma Fatty Acids

Fatty acid concentrations were measured using a direct saponification and methylation method, which was previously described (Walchuk et al., 2022). Briefly, 50µl of plasma was saponified with 0.5M methanolic KOH in a sand bath at 100 °C for one hour, followed by methylation with 14% (w/w) BF3 in methanol. The resulting fatty acid methyl esters (FAME) were separated by gas chromatography equipped with a flame ionization detector (Bruker Corp., CompassXport 3.0, Massachusetts, USA) and a BPX70 micro-column (SGE Analytical Science, Carrboro, NC, USA). Detailed run conditions have been published previously (Wang et al., 2021). The fatty acid peaks were identified using the FAME 461 standard (Nucheck Prep Inc., Waterville, Minnesota, USA).

Diabetes-associated Markers

Advanced Glycation End-Product

Advanced glycation end-products (AGE), an indicator of diabetes and cardiovascular risks (Hirano et al., 2014), were measured non-invasively as skin autofluorescence at the inner side of the dominant lower forearm using the AGE Reader (Diagnostics B.V., Groningen, The Netherlands). AGE is expressed in arbitrary units, and the score for each participant was graphed compared to the mean value as a function of age ± 1 SD.

Blood Pressure

Both diastolic and systolic blood pressure were measured using the BPTru automated device (VSM MedTech Ltd, Coquitlam, British Columbia, Canada). Participants were instructed to remain seated in a relaxed state, with their non-dominant arm resting parallel to their body on a flat surface and their legs uncrossed. The circumference of the arm at the midpoint between the shoulder and the elbow was measured to determine the appropriate cuff size. The blood pressure cuff was then placed on the non-dominant arm, centred over the brachial artery. Three measurements were automatically taken at one-minute intervals and averaged.

Pressure-Mediated Reflection Spectroscopy

Skin carotenoid status was assessed to relate to the MPOD using the Veggie Meter® (Longevity Link Corp., Salt Lake City, UT, USA), which uses pressure-mediated reflection spectroscopy to quantify skin carotenoids on a scale of 0 to 800. Higher scores indicate greater skin carotenoid levels, suggesting a higher intake of carotenoid-rich fruits and vegetables (Jilcott-Pitts et al., 2023). This method has been validated as a reliable estimate of plasma carotenoid concentrations (Jilcott-Pitts et al., 2018). Prior to each assessment, the Veggie Meter® was calibrated, and participants were instructed to wash their hands with warm water and soap and dry their hands with a disposable towel. The ring finger (i.e., fourth digit) of their non-dominant hand was placed into the device, with the pad of their finger lying flatly on the bulb. Once in place, a lever was lowered onto the finger to apply gentle pressure, ensuring an accurate reading. Measurements were performed in triplicate, and the average reading was used in the analyses. Participants removed their fingers between readings.

Body Composition

Body composition, particularly body adiposity, a risk factor for T2DM (Gómez-Ambrosi et al., 2011), was measured using the InBody570 (InBodyCo, Cerritos, United States. Prior to the assessment, height, without shoes, was measured to the nearest 0.1 cm, using a stadiometer (SECA, Hamburg, Germany). Participants were encouraged to use the restroom before testing. The height, age, and sex of participants were entered into the device. During testing, participants removed their shoes and socks, cleaned the surfaces of their hands and feet with a sanitary wipe, and then connected to the electrodes on the platform and handles. Resistance and electrical reactance were then calculated through the electrical passage between the electrodes.

Questionnaire

Self-reported data on demographics, vision, health, and nutritional status were obtained using the validated 'Nutrition and My Retina' as we have published (Walchuk et al., 2017). The vision questionnaire included was from the validated 'National Eye Institute (NEI) Visual Function Questionnaire (Mangione et al., 2001). The question breakdown is as follows: 10 questions on demographics, 17 questions on health, 10 questions on vision, and six questions on diet. The questions were multiple-choice, and the data were transcribed by code into an Excel database for statistical analysis.

Three-day Food Records

Self-reported dietary intake was collected using a Three-Day Dietary Food Record for each participant. Participants were instructed to record all food and beverages consumed over two weekdays and one weekend day within the same week. Verbal and written instructions were provided to the participants regarding serving sizes and the level of specificity required for

proper completion of the dietary record. Dietary analysis was conducted using Food Processor (Trustwell, Salem, United States), and the nutrient averages from the three days were reported.

Statistical Analysis

Data were presented as means and standard deviations (SD). Significant differences between groups were assessed by t-test for continuous variables ($p<0.05$) and a Chi-square test ($\chi 2$) for categorical variables, using the SAS 9.4 software (SAS Institute Inc.). Results were considered statistically significant at $p < 0.05$. The Spearman rank correlation coefficient (rs) was used to test the correlation between MPOD and Veggie Meter scores. The sample size for this study was calculated based on a previous study on retinal function in individuals with and without diabetes (Brooks et al, 2022). This study aimed to detect a 3.7% difference in implicit time and 27.9% in amplitude, which yielded required sample sizes of n=30 and n=12, respectively, with a level of significance of 0.05 and a power of 80%. To account for a potential dropout rate of 10%, the target was set at 33 participants per group.

Results

Participant Demographics and Self-Assessed Health and Vision Status

The characteristics of both participant groups are shown in **Table 2-1**. The average age in the NGM group was 55.2 ± 13.0 years (range 31-79), comprising 10 males (33%) and 20 females (67%). In the T2DM group, the age was 60.6 ± 11.6 years (range 29-80), with 19 males (56%) and 15 females (44%) in the T2DM group. There was no difference in age between the groups. Although no significant differences in body composition were identified, 43% of participants in the NGM group and 53% in the T2DM group were classified as obese class I, with both groups showing a high body fat percentage (37.8%). The T2DM group had significantly higher plasma glucose concentrations ($p<0.0001$) and AGE scores ($p<0.05$) compared to the NGM group.

The self-assessed physical health of the T2DM group was significantly worse than the NGM group ($p < 0.01$), with 47 % of T2DM participants reporting "poor" or "fair" health, compared to 30% in the NGM group.

Both groups reported similar vision statuses, with 43%-45% of participants reporting their vision status as "good", "very good", or "excellent", and 13-16% expressing concern about their eyesight "most" or "all of the time". Additionally, most participants (83-93%) in both groups "somewhat" or "strongly" agreed that the food they consume affects eye health.

Self-Reported Diet Intake

Due to significant differences in dietary intake among men and women, the macronutrient composition for both sexes in each group is reported separately in **Tables 2-2 and 2-3**. Overall, macronutrient intake patterns of both sexes were similar between groups. Both sexes in the NGM group fell within the acceptable macronutrient distribution ranges (AMDR) carbohydrates (46-46.9% of total kcal) and protein (15.7-16.5 % of total kcal) (Ahmed et al., 2021), but 2-3% higher than the AMDR for fat (37.3-38.2% of total kcal). In the T2DM group, both sexes were within the AMDR for protein, but exceeded the recommendations for fat (39.6-43.1% of total kcal), and fell below the recommendation for carbohydrates (40-42.7%).

Retina Function

The photopic (cone cell function) retina responses of the participants are presented in **Figure 2-1**. The T2DM group had significantly ($p<0.05$) delayed photopic implicit time (27.12 ± 1.82 ms) compared to the NGM group (25.71 ± 1.28 ms). There was no significant difference in the photopic maximum amplitude between groups.

The scotopic (rod cell function) retinal responses of the participants are presented in **Figure 2-2**. The T2DM group had significantly ($p<0.05$) delayed scotopic (b-wave) implicit time (49.84 ±

6.36 ms) compared to the NGM group (48.25 ± 3.95 ms). No significant differences were found in scotopic a-wave and b-wave maximum amplitude and a-wave implicit time. There were no significant differences in oscillatory potential implicit time and maximum amplitude between the two groups (**Figure 2-3**).

Plasma Biochemistry

Plasma lipid profiles were measured in both the T2DM and NGM groups (**Table 2-4**). The T2DM group had significantly lower plasma cholesterol ($p<0.001$), LDL ($p<0.001$), and HDL ($p<0.0001$) compared to the NGM group. As expected, the T2DM group had significantly higher plasma glucose ($p<0.0001$) compared to the NGM group. No differences were identified in TAG and hs-CRP between groups.

Plasma Fatty Acids

To investigate the relationship between fatty acids and retinal health, plasma fatty acids were measured in both the T2DM and NGM groups (**Table 2-5**). The T2DM group had significantly higher monounsaturated fatty acids (MUFA; $p<0.005$) and significantly lower plasma omega-6 polyunsaturated fatty acids (n-6 PUFA) ($p<0.005$) compared to the NGM group. The T2DM group had a significantly lower PUFA to saturated fatty acids (SFA) ratio ($p<0.02$) compared to the NGM group. No significant differences were found in plasma saturated fatty acids, total omega-3 PUFA, docosahexaenoic acid (DHA), and α-linolenic acid (ALA) between the T2DM and NGM groups.

Carotenoid Status and Macular Pigment

Carotenoid status (**Figure 2-4**) was measured to correlate with MPOD. The T2DM group had significantly lower skin-based carotenoid scores compared to the NGM group (235 ± 73 and 272 ± 63, respectively; $p<0.05$).

Absolute (central and peripheral) and estimated (central only) MPOD are displayed in **Figure 2-5**. While both groups fall within the range of 0.21-0.5, which is classified as inadequate, the T2DM group exhibited lower estimates (10.5%) and absolute (7.3%) MPOD values compared to the NGM group, with no significant differences observed. No significant correlations existed between absolute and estimated MPOD and carotenoids in both groups (**Table 2-6**).

Discussion

The present study aimed to examine retinal function, macular pigment density, and associated plasma factors in individuals diagnosed with T2DM without diabetic retinopathy. The principal finding was that, despite no significant differences in self-reported vision health, participants with T2DM exhibited significantly slower light-stimulated retinal responses compared to non-diabetic individuals under free-living conditions. This finding highlights a potential discrepancy between subjective perception and objective measurements of retinal function, underscoring the importance of regular retinal assessments for the early detection and prevention of diabetic retinopathy. Moreover, the T2DM group has lower macular pigment density, which is responsible for protecting the retina from oxidative stress, compared to the NGM group. However, this difference was not significant.

Self-Reported Vision Health

There were no significant differences in perceived vision status between individuals with T2DM and the age-matched NGM group in the current study, despite objective retina measurements with ERG showing differences between the groups. It is known that functional and structural abnormalities in retinal cells precede the visual diagnostic symptoms that characterize the early stages of diabetic retinopathy (Kusuhara et al., 2018). Qaseem et al. (2020)

found that individuals in the early stages of diabetic retinopathy were often unaware of their condition, underestimating risk to their vision. Furthermore, those with more advanced disease frequently underestimate its severity. Individuals with T2DM are at risk of developing diabetic retinopathy; the current study participants self-reported comparable vision status to that of individuals without diabetes. Overall, individuals with T2DM showed no greater concern for their eyesight than those in the NGM group, despite being at a higher risk for developing retinopathy.

Retina Function

In this cross-sectional study, individuals with T2DM, with duration ranging from <1 year to 22 years, exhibited significantly delayed scotopic b-wave and photopic implicit time responses compared to age-matched individuals with NGM. This finding suggests some abnormalities in the bipolar cell responding to the onset of light in the inner retina (Eggers & Carreon, 2021). Lecleire-Collet et al (2011) also found that participants with T2DM had significantly delayed scotopic a-wave and b-wave implicit times. They also found significantly reduced scotopic b-wave maximum amplitude in their study participants, although we did not observe a difference in amplitude.

We found no significant differences in OP implicit time and maximum amplitude between our two groups. This is inconsistent with most research, which shows that dark-adapted OP implicit time and maximum amplitude are often abnormal in the early stages of diabetes and worsen as the disease progresses. Indeed, Chi et al (2010) found significantly delayed OP implicit time and lower OP maximum amplitude in T2DM participants without retinopathy, but no differences in photopic and scotopic implicit time and maximum amplitude responses. These discrepancies may be due to Chi et al.'s small sample size (n = 15) and the unspecified duration

of diabetes, both of which are important limitations, as disease duration influences retinal changes. This discrepancy in the current study can be partly explained by the lack of minimum criteria for diabetes diagnosis. Some of our participants in the T2DM group were recently diagnosed and, therefore, damage to the retinal cells from the elevated glucose levels and inflammation from diabetes may not be present. Similarly, the plasma glucose analysis revealed that six participants (20%) in our NGM group had plasma glucose levels between 5.6 and 6.9 mmol/L, indicating a pre-diabetic condition. The damage to the retinal cells caused by high plasma glucose concentrations and inflammation may have affected the results.

Macular Pigment Density and Carotenoids

The present study found that the macular carotenoid levels measured by MPOD were lower in our T2DM group compared to the NGM group (0.41 and 0.44, respectively), with no significant differences. The value of MPOD is in a similar range to other studies, which reported an average score of 0.45 in the diabetic group without retinopathy (She et al., 2016). However, a comparative study found participants with T2DM, with or without retinopathy, had significantly reduced MPOD compared to the non-diabetic control group (Lima et al, 2010). The discrepancy in our study could, in part, be explained by the recent diagnosis of some study participants (<6 months). Previous research has shown that several factors can contribute to low MPOD, including hyperglycemia, oxidative stress, and body fat (She et al., 2016); therefore, a larger sample size is needed to stratify the groups. Ample studies support the protective role of MPOD in age-related macular degeneration; however, more research is needed to support its role in diabetic retinopathy (Arunkumar et al., 2018).

The present study measured skin-based carotenoid status using the Veggie Meter, which is 80-96% correlated with plasma carotenoids tested in five diverse human cohorts of various

ages and ethnicities (n = 146) (Ermakov et al., 2016). The T2DM group had significantly lower skin-based carotenoid scores compared to the NGM group. However, both study groups had inadequate carotenoid levels, which are important for maintaining eye health. Adequate intake corresponds to a Veggie Meter score between 280 and 480, with one serving of carotenoid-rich fruits and vegetables equating to approximately 100 units on the Veggie Meter® scale (Rush et al., 2019). Our findings are consistent with other research that found similar average Veggie Meter scores in Hispanic, Italian, and Dominican populations (Di Noia & Gellermann, 2021; Augimeri et al., 2024). The low skin-based carotenoid score in both groups reflects the inadequate intake of carotenoid-rich fruits and vegetables, a common trend among all Canadians (Augimeri et al., 2024). Our findings are particularly concerning for our study population, considering carotenoid-rich foods provide beneficial antioxidants for maintaining eye health, and individuals with T2DM have an increased risk for developing retinopathy (Tuj Johra et al., 2020).

We raised the question of whether plasma carotenoid status is correlated with macular carotenoids. We found no significant correlation between skin-based carotenoids and MPOD in either of the study groups. She et al. (2016), with a large sample size (n = 435), found a positive association between MPOD level and higher intake of carotenoid-rich foods among participants with diabetes. Unfortunately, our study did not measure direct carotenoid intake levels. A recent observational study conducted by Smith et al. (2025) found that skin carotenoids accounted for approximately one-third of the variance in retinal carotenoid density. Since we are the first study to report correlation findings between skin-based carotenoids, as measured by the Veggie Meter®, and MPOD in a group with T2DM, a further study is necessary to establish a robust relationship between these two parameters in a larger sample size.

Plasma Parameters

In the present study, plasma lipid parameters in the T2DM group were significantly more favourable, with lower plasma cholesterol and LDL than those in the NGM group. This finding was unexpected, but a more plausible explanation for the observed difference is that approximately 30% of the T2DM group was taking lipid-lowering medications, compared to only 3% in the NGM group. This is consistent with previous research showing that individuals with T2DM are more likely to use lipid-lowering medications due to the increased risk of cardiovascular disease (Karlsson et al., 2018). Although the T2DM group had significantly higher intake of fat, it did not reflect TAG level in the plasma, indicating more favourable roles of medications in lipid profiles observed in the T2DM group (Zhang et al., 2020).

Conclusion

In conclusion, individuals with T2DM in the free-living conditions, diagnosed <1 and 22 years, had poorer retinal cell function despite no self-perception of vision issues. Both groups had an inadequate level of MPOD (0.21-0.5), which may lower antioxidant protection in the retina. Considering that both individuals with T2DM and NGM had poor diet quality, the public health messaging on healthy diets needs to continue for all Canadians. Additionally, dietary education programs for individuals with T2DM should include specific foods and nutrients beneficial to the eyes in order to reduce the risk of developing retinopathy. Finally, future clinical research should be conducted to test eye-beneficial nutrients in individuals with T2DM and without diabetic retinopathy using electroretinograms.

Acknowledgements

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Author Contributions

CW., JG., and MS., participated in the conceptualization and design of the study; JG. HB, and MS oversaw the study execution; JG. and JC conducted participant recruitment and sample and data collection; JC and CW performed the biochemical analyses; JC and SK conducted the statistical analyses; JC drafted the manuscript; JG and MS reviewed and edited the manuscript. All authors have read and agreed to the published version of this manuscript. MS is responsible to provide overall guidance and financial support for this study.

Table 2-1. Characteristics and self-perceived heath and vision status of participants with and without type 2 diabetes

Variables	Response	NGM (n=30)	T2DM (n=34)	p-value
Age (years)		55.2 ± 13.0	60.6 ± 11.6	NS
Sex, *n* (%)				
	Male	10 (33.3)	19 (55.9)	NS
	Female	20 (66.7)	15 (44.1)	
Height (cm)		167.9 ± 7.8	170.0 ± 8.7	NS
Weight (kg)		87.1 ± 22.4	90.6 ± 20.6	NS
BMI (kg/m^2)		31.0 ± 8.6	31.6 ± 7.0	NS
	Underweight (<18.5)	0	0	
	Normal (18.5-24.9)	33.3	16.7	
	Overweight (24.9-29.9)	23.3	26.7	
	Obese (>30)	43.3	56.7	
Body Fat (%)	Men	34.9 ± 10.5	36.6 ± 10.1	NS
	Women	39.2 ± 11.5	40.2 ± 9.9	
Systolic BP (mmHg)		133.2 ± 18.5	128.7 ± 14.2	NS
Diastolic BP (mmHg)		83.2 ± 10.9	80.2 ± 8.8	NS
Plasma Glucose (mmol//L)		5.39 ± 0.55	8.79 ± 3.15	<0.01
AGEs		2.3 ± 0.6	2.7 ± 0.7	<0.05
Self-assessed physical health (%)				<0.01
	Poor	3.0	10.0	
	Fair	26.7	36.7	
	Good	40.0	53.3	
	Very Good	26.7	0.0	
	Excellent	0.0	0.0	
Self-ranked vision status (%)				NS
	Poor	16.7	0.0	
	Fair	26.7	44.0	
	Good	36.7	32.0	
	Very Good	3.3	12.0	
	Excellent	3.3	0.0	

Values are mean ± SD or percentage (%) for each group Significant differences between groups were testes by t-test for continuous variables ($p<0.05$) and a Chi-square test ($\chi 2$) for categorical variables.

Abbreviations: AGEs, advanced glycation end products; BMI, body mass index; NS, not significant. NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

Table 2-2. Macronutrient composition of participants with and without type 2 diabetes

	NGM		T2DM			
	Male (n=8)	Female (n=22)	Male (n=18)	Female (n=12)	*p*-value[1]	*p*-value[2]
Calories (kcal)	1995.6 ± 209.6	1660.3 ± 85.2	2475.0 ± 197.5	1696.7 ± 129.9	NS	NS
Carbohydrate (g)	229.7 ± 20.8	194.8 ± 13.4	264.5 ± 25.6	169.5 ± 17.3	NS	NS
Fibre (g)	19.5 ± 1.8	15.9 ± 1.1	19.7 ± 1.9	15.6 ± 2.0	NS	NS
Sugars (g)	77.1 ± 10.3	75.4 ± 6.3	93.2 ± 16.4	55.0 ± 8.4	NS	NS
Protein (g)	82.3 ± 13.2	65.0 ± 2.5	103.5 ± 7.5	68.3 ± 3.7	NS	NS
Fat (g)	84.6 ± 10.9	68.9 ± 3.7	108.9 ± 10.2	80.1 ± 9.3	NS	NS
Saturated Fat (g)	27.0 ± 3.8	23.3 ± 1.5	34.9 ± 3.6	24.7 ± 2.1	<0.001	NS
Trans Fat (g)	0.4 ± 0.1	0.4 ± 0.1	0.9 ± 0.2	0.3 ± 0.1	NS	NS
Cholesterol (mg)	436.3 ± 39.9	398.9 ± 15.5	546.8 ± 20.1	445.8 ± 24.1	<0.05	NS

Values are mean ± SD. Significant differences in each sex between groups were testes by t-test ($p<0.05$).
[1] represents significance differences between male participants.
[2] represents significance differences between female participants.
Abbreviations: NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

Table 2-3. Macronutrient AMDR of participants with and without T2DM

	NGM		T2DM			
	Male (n=8)	Female (n=22)	Male (n=18)	Female (n=12)	*p*-value[1]	*p*-value[2]
Carbohydrate (%)	46.0	46.9	42.7	40.0	NS	NS
Protein (%)	16.5	15.7	16.7	16.1	NS	NS
Fat (%)	38.2	37.3	39.6	43.1	NS	NS

Values are mean ± SD. Significant differences in each sex between groups were tested by t-test ($p<0.05$).
[1] represents significance differences between male participants.
[2] represents significance differences between female participants.
Abbreviations: NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

Table 2-4. Plasma blood profile of participants with and without type 2 diabetes

	NGM (n=25)	T2DM (n=29)	*p*-value
GLC (mmol/L)	5.39 ± 0.55	8.79 ± 3.15	<0.01
TAG (mmol/L)	1.36 ± 0.55	1.78 ± 1.02	NS
CHOL (mmol/L)	5.15 ± 0.82	4.31 ± 1.01	<0.001
LDL (mmol/L)	2.98 ± 0.74	2.32 ± 0.98	<0.01
HDL (mmol/L)	1.41 ± 0.29	1.08 ± 0.24	<0.01
CRP (mg/L)	2.56 ± 2.87	3.53 ± 3.52	NS

Values are mean ± SD; Significant differences between groups were tested by t-test ($p<0.05$). GLC, glucose; TAG, triglycerides; CHOL, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; CRP, high-sensitivity C-reactive protein; NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

Table 2-5. Plasma fatty acids of participants with and without type 2 diabetes

(%, w/w)	NGM (n=27)	T2DM (n=30)	p-value
ΣSFA	33.55 ± 1.93	34.06 ± 2.54	NS
ΣMUFA	26.24 ± 3.61	29.23 ± 3.86	p<0.01
Σn-6 PUFA	34.96 ± 4.35	31.52 ± 4.39	p<0.01
Σn-3 PUFA	3.63 ± 0.63	3.66 ± 0.70	NS
DHA	1.43 ± 0.39	1.53 ± 0.47	NS
ALA	0.70 ± 0.21	0.71 ± 0.23	NS

Values are mean ± SD; Significant differences between groups were tested by t-test (p<0.05). ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; Σn-6 PUFA, total omega-6 fatty acids; Σn-3, total omega-3 fatty acids; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

Table 2-6. Correlation between veggie meter score and MPOD of study participants with and without type 2 diabetes

	Group	Veggie Meter Score	
Estimated MP	NGM	0.1784	0.0089
Absolute MP	T2DM	0.1142	0.2515

Values are expressed as Spearman's correlation coefficient (r_s); * Correlation is significant at $p < 0.05$.

Abbreviations: MP, macular pigment; NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

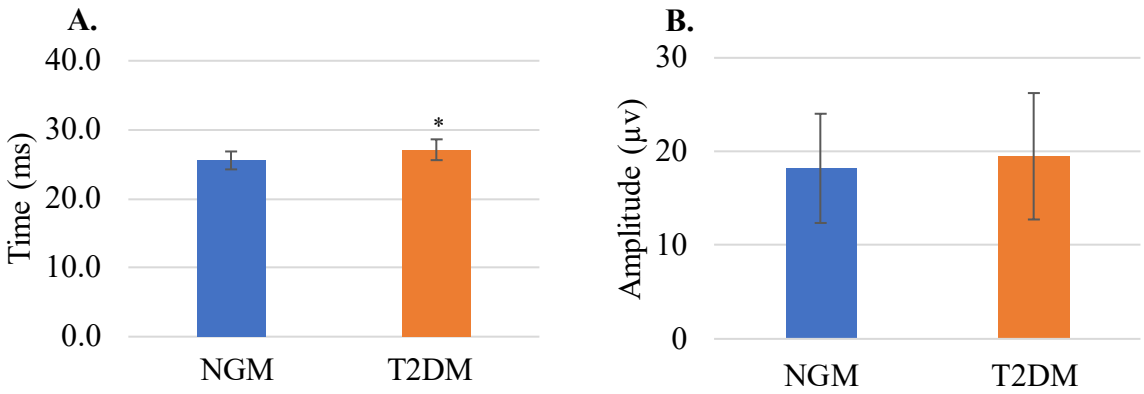

Figure 2-1. Photopic (light-adapted) responses of study participants with and without type 2 diabetes (A) implicit time (msec); (B) maximum amplitude (µv). Results expressed as mean ± SD (n= 29-33). Data were analyzed using independent t-test.
*, significantly different compared to NGM group ($p<0.05$). Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

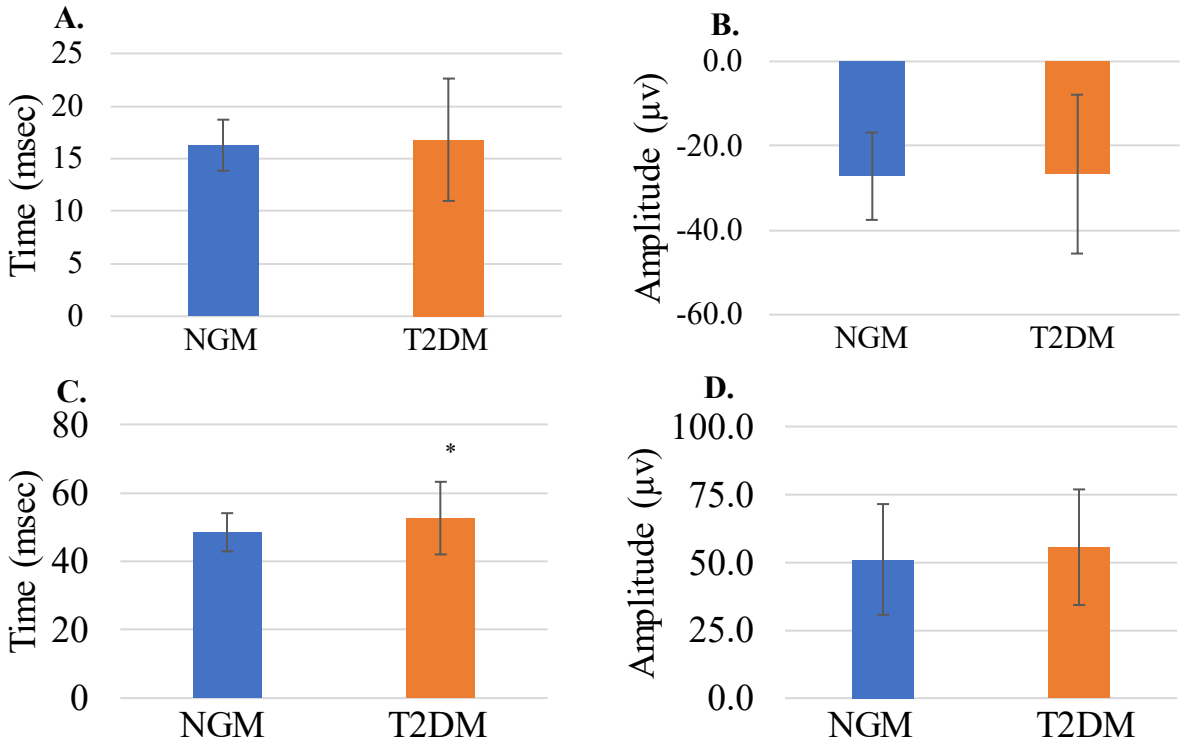

Figure 2-2. Scotopic (dark-adapted) responses of study participants with and without type 2 diabetes (A) a-wave implicit time (msec); (B) a-wave maximum amplitude (µv); (C) b-wave implicit time (msec); (D) b-wave maximum amplitude (µv). Results expressed as mean ± SD (n= 27-32). Data were analyzed using independent t-test. *, significantly different compared to NGM group ($p<0.05$). Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

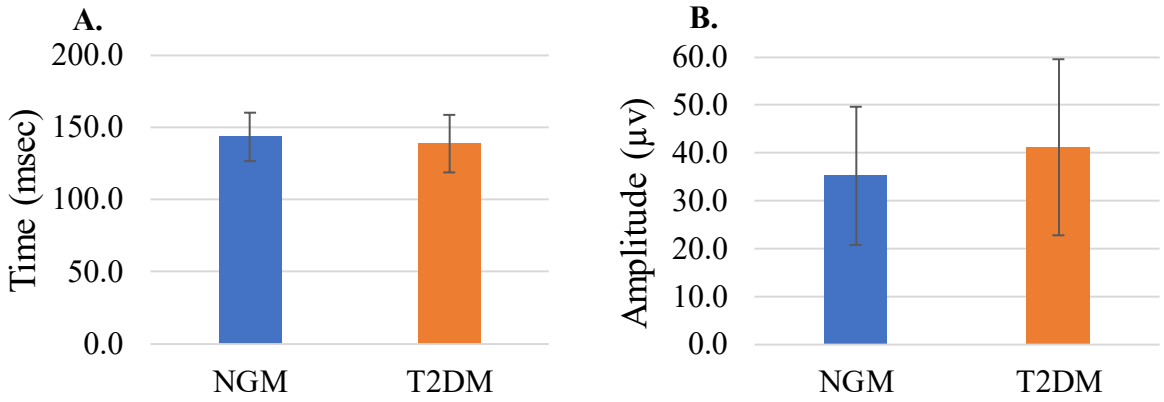

Figure 2-3. Oscillatory potential response (inner retina neuron) responses of study participants with and without type 2 diabetes (A) implicit time (msec); (B) maximum amplitude (µv). Results expressed as mean ± SD (n= 25-30). No significant differences were found.
Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

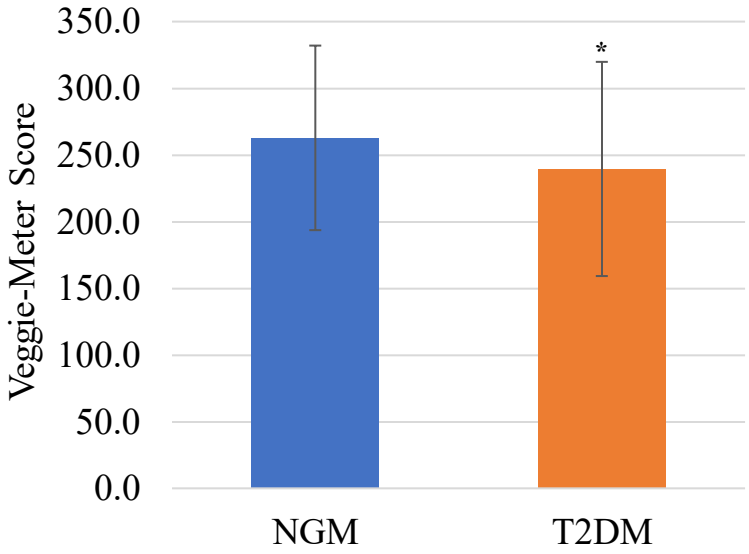

Figure 2-4. Skin-based carotenoid status responses of study participants with and without type 2 diabetes. Results expressed as mean ± SD (n= 29-33). Data were analyzed using indeppendent t-test. *, significantly different compared to NGM group ($p<0.05$). Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

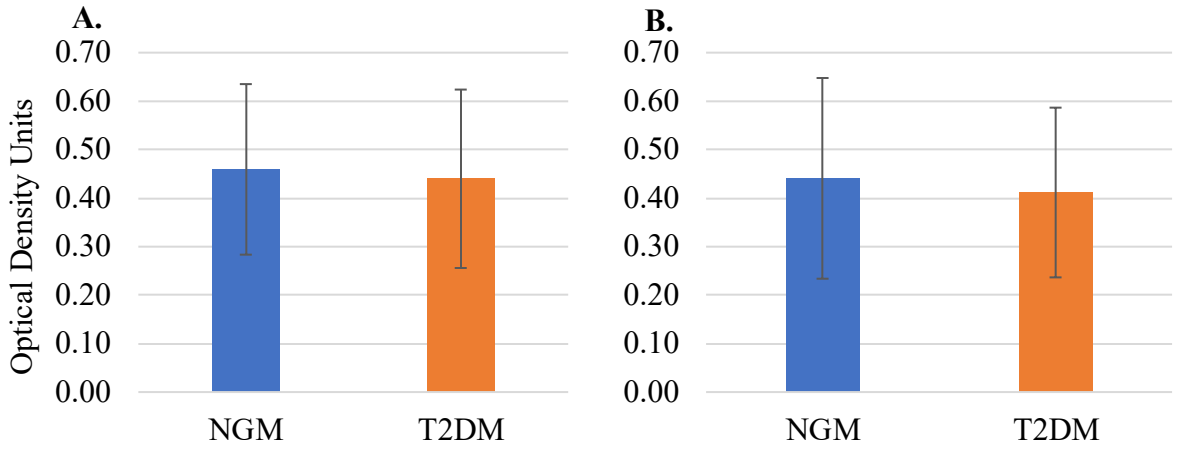

Figure 2-5. Macular pigment optical density of study participants with and without type 2 diabetes. (A) absolute (combined central and peripheral vision) MPOD; (B) estimated (central vision only) MPOD. Results expressed as mean ± SD (n= 27-32). Data were analyzed using indeppendent t-test. No significant differences were observed.
Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

Chapter 3. Effects of DHA and lutein enriched eggs on retina health and metabolic parameters in individuals with type 2 diabetes

Abstract

Lutein and docosahexaenoic acid (DHA) are key nutrients for eye health, supporting macular pigment and retina, respectively. Consumption of eggs enriched with lutein and DHA has been shown to positively influence retinal function in healthy older Caucasian adults. This study investigated the effect of consuming lutein- and DHA-enriched eggs on retinal health and metabolic parameters in individuals with type 2 diabetes (T2DM). A total of 60 participants (58 ± 13 years old), including 30 participants with T2DM and 30 age-matched participants with normal glucose metabolism (NGM), consumed two enriched eggs (0.87 mg lutein/day, 220 mg DHA/day) daily for 6 weeks. Retina health was assessed using a full-field electroretinogram, macular pigment optical density and self-reported vision status. Plasma metabolic markers, including DHA and fatty acid profiles, were also analyzed. Additional assessments included skin-based carotenoid score, advanced glycation end products, vascular stiffness, and body composition. All assessments were performed at baseline (PRE, week 0), midpoint (DURING, week 3), and after the egg intervention (POST, week 6). Enriched egg consumption for 6 weeks did not alter retinal function or macular pigment levels; however, significant differences were observed between T2DM and NGM groups in photoreceptor cell implicit time, with slower response to light in individuals with T2DM ($p<0.05$). Additionally, individuals with T2DM had significantly lower skin-based carotenoid scores compared to their NGM counterparts ($p<0.05$). Plasma DHA levels increased after 3 weeks of egg consumption ($p<0.05$) and remained elevated throughout the study, with no differences between groups. Daily consumption of the enriched eggs did not adversely affect plasma lipid profiles, glucose concentration, body weight and composition, or vascular parameters in either group. Overall, daily intake of two lutein- and DHA-enriched eggs for 6 weeks did not impact retinal health but revealed distinct differences in health and vision parameters between individuals with and without T2DM. Future studies should explore longer intervention periods or compare these effects to those of regular eggs.

Keywords: Electroretinogram, Egg, Diabetic Retinopathy, Type 2 Diabetes, Docosahexaenoic Acid, Lutein, Macular pigment, Retina

Introduction

Diabetic retinopathy is one of the most common and serious long-term complications associated with diabetes. The vast majority (99%) of individuals with type 1 diabetes will develop some degree of retinopathy throughout their lives, as will about 60% of individuals with type 2 diabetes (Klein et al., 2008). Although optimal management, such as tight control of glucose and blood pressure, treatment of dyslipidemia, and smoking cessation, reduces the risk, about 12% of individuals still develop retinopathy (Canadian Diabetes Association, 2024). Timely studies to establish preventative strategies for managing microvascular complications of the retina are essential for maintaining and improving the quality of life.

The retina, a light-sensitive tissue lining the inner surface of the eye, is composed of neurons connected by synapses (Mahabadi & Khalili, 2023). Its molecular composition contains the highest concentration of the omega-3 fatty acid docosahexaenoic acid (DHA) in the body, and rich in phospholipids that contain both DHA and very long chain fatty acids (VLCFA) (Suh et al., 2009; Zemski-Berry et al., 2014; Sugasini et al., 2020). A deficiency in these fatty acids can lead to abnormal retinal function and structure. This has been demonstrated in both transgenic mice and humans lacking the ELOVL4 (elongation of very long chain fatty acid) gene, resulting in retinal dystrophies (Kuny et al., 2014; Dornstauder et al., 2012; Nwagbo & Bernstein, 2023). These fatty acids are, thus, considered critical regulators for visual cell function and potential therapeutic targets for the treatment of retinal diseases.

The yellow spot near the center of the retina is composed of lutein and zeaxanthin, collectively referred to as the macular pigment. These compounds act as potent antioxidants and serve as natural filters for high-energy blue light, offering protection against light-induced retinal damage (Mrowicka et al., 2022). Because diabetic retinopathy is thought to be associated with

long-term oxidative damage to the retina (Kowluru & Chan, 2007), these carotenoids may help prevent this disease. The retina's constant exposure to light throughout the lifetime, combined with its high content of unsaturated lipids (Jarrett & Boulton, 2012), makes it especially vulnerable to oxidative damage unless properly balanced with antioxidants.

Research has shown that the above molecular composition in the retina can be modulated through dietary interventions. Eggs, in particular, are a widely available and cost-effective source of essential nutrients such as choline, unsaturated fatty acids, essential amino acids, carotenoids, and various vitamins and minerals (Walchuk et al., 2022). Several of these nutrients play a key role in maintaining ocular health. A recent study found that daily consumption of two medium lutein and DHA enriched eggs per day (~870µg lutein and ~220mg DHA) over 6 weeks positively influenced retina function among healthy older Caucasian adults, without negatively affecting blood lipid profiles (Walchuk et al., 2022). It remains unclear whether similar effects can be found in individuals at risk of diabetic retinopathy.

Therefore, this study was planned to evaluate the effects of consuming lutein- and DHA-enriched eggs on electrophysiological retinal function, macular pigment optical density, and key metabolic parameters. Additionally, the study assessed the impact of enriched egg consumption on self-reported vision and overall health status, levels of advanced glycation end products, arterial stiffness, and body composition. Finally, the study aimed to compare retinal and cardiometabolic outcomes between individuals with type 2 diabetes and age-matched individuals with normal glucose regulation.

Experimental Design and Measurement

Participants and Study Design

Participant

A total of 60 participants, including 30 men and women (age >19 years old) with type 2 diabetes (T2DM) and 30 age-matched individuals with normal glucose metabolism (NGM), were recruited to take part in this clinical trial at the I.H. Asper Clinical Research Institute, Winnipeg, Manitoba, Canada. This design included a dietary adaptation period (3 weeks) between the first two visits to reduce internal variations among participants before beginning the experimental period with egg consumption. The participants were instructed to maintain their routine diets while avoiding eggs and nutritional supplements (i.e., DHA, omega-3, carotenoids, choline and lutein).

Inclusion criteria for the T2DM group were male or female over the age of 18; previous diagnosis of T2DM, thus currently taking oral hypoglycemic medications. Inclusion criteria for the NGM group included male or female over the age of 18; not currently diagnosed with pre-diabetes or T2DM; not taking oral hypoglycemic medications. Both T2DM and NGM groups have not donated blood in the past two months and are willing not to donate for the duration of this trial; has not in the last month participated in another dietary intervention trial and are willing not to start one for the duration of this study; and willingness to comply with the protocol requirements and provide informed consent.

Exclusion criteria for the T2DM and NGM groups were previous diagnosis with diabetic retinopathy; previous diagnosis of eye disease, mental cognitive disease, type 1 diabetes; taking insulin; diagnosed with cancer in the last 5 years; currently (within the past 3 months) diagnosed with anemia; currently pregnant or breastfeeding; taking a multivitamin, DHA and/or mineral supplements that contain vitamin A, lutein, zeaxanthin, omega-3, DHA, zinc or beta-carotene within 30 days prior to study enrollment or during; a bacterial, viral, or fungal infection within 30 days prior to study enrolment; any medical conditions or surgical interventions within 3

months of the first study visit; inability to take prescribed medications without food; inability to fast overnight; and sensitivity to flashing lights.

All participants were asked to provide written informed consent prior to participation in the study. Participants who provided written consent were assessed over the phone for their eligibility for the NGM or T2DM groups.

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the University of Manitoba Research Ethics Board (HS23602, B2020:007) and the St. Boniface Hospital Research Ethics Committee (RRC/2020/1964). All participants provided written informed consent consistent with guidelines for the protection of human research participants. The study protocol was registered on ClinicalTrials.gov (NCT04496817).

Study Design

This study was a single-site, prospective clinical study. Each participant completed four visits: a screening visit, baseline (PRE, week 0), midpoint (DURING, week 3), and after (POST, week 6) of egg intervention, with each visit separated by 3 weeks. At the screening visit, the participants were instructed to maintain their routine diets while avoiding eggs, foods high in eye-beneficial nutrients, and nutritional supplements (DHA, carotenoids, lutein, and choline). This was followed by a 6-week dietary intervention where participants consumed two DHA- and lutein-enriched eggs (250mg of DHA and 1mg of lutein) per day. Fasting plasma, retina function (full field electroretinography), macular pigment optical density (MPOD, heterochromatic flicker photometry), body weight, height, BMI, body composition (bioelectrical impedance analysis), blood pressure, arterial stiffness (pulse wave analysis), skin-based carotenoid status (pressure-mediated reflection spectroscopy), and a self-reported questionnaire (*Nutrition and My Retina*)

on social-demographics, health, nutrition, and visual function were obtained at PRE, pre-intervention), DURING and POST assessments. Three-day food records were also obtained at PRE, DURING, and POST assessments.

Egg Intervention

The eggs used in this study were supplied as an in-kind donation from Burnbrae Farms Ltd. (Winnipeg, Manitoba, Canada). The medium (49-55g) DHA- and lutein-enriched eggs are commercially available as 'Naturegg Omega Plus Eggs'. Hens are fed fish oil to produce eggs containing 125 mg of DHA per 53g egg and alfalfa and corn to increase lutein concentrations to 0.5 mg per 53g egg (Walchuk et al., 2022; Burnbrae Farms Ltd.). The participants in both T2DM and NGM groups were asked to consume two medium-sized enriched eggs daily for at least 5 days a week for the duration of the study, as we published previously (Walchuk et al., 2022). Fresh eggs were provided on two occasions to the participants: at PRE and DURING visits.

Study Procedures and Measurements

The procedures and measurements used in this study were consistent with those outlined in Chapter 2.

Retina and Macular Health

Electroretinogram

Changes in retinal function were ascertained by measuring parameters related to the ffERG waveform in response to flashing light stimuli: time of response to light (implicit time) and the strength of the response (maximum amplitude) (McAnany et al., 2022). The full-field electroretinography (ffERG) was used to assess retinal cell function, including rods and cones

using the *RETeval*® (LKC Technologies, Gaithersburg, MD, USA). The ffERG responses were recorded using disposable sensor strip electrodes placed 2 mm inferior to each lower eyelid margin and aligned with the participants' non-dilated pupils, following the manufacturer's guidelines. Light-adapted, photopic response was performed using an 85 Td·s stimulus at 28.3 Hz flicker within a small 60 mm Ganzfeld dome. Both eyes were tested sequentially, starting with the right eye while the contralateral eye remained covered. Measurements were performed in duplicate. Participants then underwent a 10-minute dark-adaptation period in a light-free room.

During the dark-adapted testing, scotopic ERG testing of rod cells was performed under a dim red-light illumination with the *RETeval*® device delivering intermittent flashes with an intensity of 85 Td·s, at 0.1 Hz flicker. Eye position stability was instructed by encouraging fixation on a red central dot inside the stimulator, with fixation monitored by viewing the pupil through the *RETeval*® device. Both eyes were tested sequentially, and measurements were performed in duplicate.

The ERG recording produces a biphasic waveform plotted on a chart, with the X-axis representing the response time (implicit time) in milliseconds and the Y-axis representing response strength (amplitude) in microvolts (McAnany et al., 2022). The waveform consists of three main components: the a-wave, the b-wave, and the oscillatory potentials (OPs). The a-wave reflects outer retinal function, primarily from rod and cone photoreceptors. The b-wave corresponds to activity in the inner retinal Müller and bipolar cells. Finally, the OPs represent the electrical activity of amacrine cells. Under light-adapted conditions, the signal flow primarily involves cone photoreceptors transmitting to the inner retina, while under dark-adapted conditions, the signal flow is dominated by rod photoreceptors.

A total of 62 participants completed the photopic ERG assessment, and 56 participants completed the scotopic ERG assessment. The scotopic assessment was considerably longer, making it challenging for participants to keep their eyes open for long enough to obtain accurate readings

Macular pigment optical density

Macular pigments were quantified in terms of macular pigment optical density (MPOD), which was measured using the heterochromatic flicker photometer Quantify MPS II system (Premier Eyecare, Knoxville, TN, USA, ZeaVision LLC, Chesterfield, MO, USA. While sitting down, the participant was asked to look into the machine with one eye and fixate a central 1 ° target. Once this central target starts to flicker, they were instructed to press a button and continue looking for the next flicker. The detailed protocol is as follows: The target in the system will alternate between a green (530nm) and blue (465nm) LED light with luminance up to 200 cd/m2. Throughout the assessment, these lights start to flicker at a rate of 60 Hz per second gradually slowing to 6 Hz per second. The blue light is absorbed by the macular pigments, while the green light is not. Initially, the target appears static, but as the flickering rate between the blue and green light slows, a flicker appears, and the participant clicks the button. Measurements are obtained from the blue and green light ratios at the time the participant clicked the button.
For peripheral vision, participants were asked to fixate on a 2° red spot located 8° horizontally from the central target. The same procedure stated above was repeated, and participants were asked to press the button when they observed a flicker in the central target using their peripheral vision. Once finished, the MPOD calculation is based on the endpoint when each participant pressed the response button and the corresponding blue and green light ratio. The MPOD score ranges from 0 to 1, with scores of 0-0.21 being considered very inadequate, 0.21-0.5 being

inadequate, 0.5-0.7 being adequate, and 0.7-1 being excellent (Van Der Veen et al., 2009). Measurements were repeated twice, and for each eye test, the contralateral eye was closed or covered.

Plasma Metabolites

Plasma Biochemistry

Fasted plasma glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, and hs-C-reactive protein were determined using the automated Roche Diagnostics Cobas c 111 Analyzer (F. Hoffman-La Roche Ltd., Basel, Switzerland).

Plasma Fatty Acids

Fatty acid concentrations were measured using a direct saponification and methylation method, which was previously described (Walchuk et al., 2022). Briefly, 50µl of plasma was saponified with 0.5M methanolic KOH in a sand bath at 100 °C for one hour, followed by methylation with 14% (w/w) BF3 in methanol. The resulting fatty acid methyl esters (FAME) were separated by gas chromatography equipped with a flame ionization detector (Bruker Corp., CompassXport 3.0, Massachusetts, USA) and a BPX70 micro-column (SGE Analytical Science, Carrboro, NC, USA). Detailed run conditions have been published previously (Wang et al., 2021). The fatty acid peaks were identified using the FAME 461 standard (Nucheck Prep Inc., Waterville, Minnesota, USA).

Diabetes Associated Risk Markers

Advanced Glycation End Products

Advanced glycation end products (AGE), an indicator of diabetes and cardiovascular risks, were measured non-invasively as skin autofluorescence at the inner side of the dominant lower forearm using the AGE Reader mu (Diagnoptics B.V., Groningen, The Netherlands). AGE is expressed in arbitrary units, and the score for each participant was graphed compared to the mean value as a function of age ± 1 SD.

Blood Pressure

Both diastolic and systolic blood pressure were measured using the BPTru automated device (VSM MedTech Ltd, Coquitlam, British Columbia, Canada). Participants were instructed to remain seated in a relaxed state, with their non-dominant arm resting parallel to their body on a flat surface and their legs uncrossed. The circumference of the arm at the midpoint between the shoulder and the elbow was measured to determine the appropriate cuff size. The blood pressure cuff was then placed on the non-dominant arm, centred over the brachial artery. Three measurements were automatically taken at one-minute intervals and averaged.

Body Composition

Body composition, particularly body adiposity, a risk factor for T2DM (Gómez-Ambrosi et al., 2011), was measured using the InBody570 (InBodyCo, Cerritos, United States. Prior to the assessment, height, without shoes, was measured to the nearest 0.1 cm, using a stadiometer (SECA, Hamburg, Germany). Participants were encouraged to use the restroom before testing. The height, age, and sex of participants were entered into the device. At the time of testing, participants removed their shoes and socks and cleaned the surfaces of their hands and feet with a sanitary wipe before connecting to the electrodes on the platform and handles. Resistance and electrical reactance were then calculated through the electrical passage between the electrodes.

Pressure-mediated Reflection Spectroscopy

Skin carotenoid status, used as an indirect measure of plasma carotenoid concentrations, was assessed using the Veggie Meter® (Longevity Link Corp., Salt Lake City, UT, USA), which employs pressure-mediated reflection spectroscopy to quantify skin carotenoid levels on a scale ranging from 0 to 800. Higher scores indicate greater skin carotenoid levels, reflecting a higher intake of carotenoid-rich fruits and vegetables (Jilcott-Pitts et al., 2023). This method has been validated as a reliable estimate of plasma carotenoid concentrations (Jilcott-Pitts et al., 2018). Prior to each assessment, the Veggie Meter® was calibrated, and participants were instructed to wash their hands with warm water and soap and dry their hands with a disposable towel. The measurement was taken from the ring finger (i.e., fourth digit) on the non-dominant hand, which was positioned flat against the bulb of the device. Once in place, a lever was lowered onto the finger to apply gentle pressure, ensuring an accurate reading. Measurements were performed in triplicate, removing their finger between each reading. The average of the readings was used in the analyses.

Questionnaire

Self-reported data on demographics, vision, health, and nutrition status were obtained using the validated 'Nutrition and My Retina' as we have published (Walchuk et al., 2017). The vision questionnaire included was from the validated 'National Eye Institute (NEI) Visual Function Questionnaire' (Mangione et al., 2001). The question breakdown is as follows: 10 questions on demographics, 17 questions on health, 10 questions on vision, and six questions on diet. The questions were multiple choice, and the data were transcribed by code into the Excel database for statistical analysis.

Statistical Analysis

The egg intervention data were analyzed using a mixed method repeated measures model (PROC MIXED) to identify significant differences between the two participant groups using SAS 9.4 software (SAS Institute Inc.). The PROC MIXED model performed comparisons among the least-squares means for both groups (NGM and T2DM) at Week 3 (PRE), Week 6 (DURING), and Week 9 (POST) time points. Tukey-Kramer was the *post hoc* test used to determine the differences between least-squares means. The Shapiro-Wilk test was used to verify the normality of the data. Results were considered statistically significant at $p < 0.05$. The Spearman rank correlation coefficient (rs) was used to test the correlations between retina function measures and plasma lipid and biochemistry profiles. The sample size for this study was calculated based on a previous study on retinal function in individuals with and without diabetes (Brooks et al, 2022). This study aimed to detect a 3.7% difference in implicit time and 27.9% in amplitude, which yielded required sample sizes of n=30 and n=12, respectively, with a level of significance of 0.05 and a power of 80%. To account for a potential dropout rate of 10%, the target was set at 33 participants per group.

Results

Participant Demographics

The characteristics of both participant groups are shown in **Table 3-1**. The average age in the NGM group was 55.2 ± 13.0 years (range 31-79), comprising 10 males (33%) and 20 females (67%). In the T2DM group, the age was 61.0 ± 11.9 years (range 29-80), with 16 males (54%) and 14 females (46%) in the T2DM group. There was no difference in age between the groups. Although no significant differences in body composition were identified, 43% of participants in the NGM group and 53% in the T2DM group were classified as obese class I, with both groups

showing a high body fat percentage (37.8%). As expected, the T2DM group had significantly higher plasma glucose concentrations ($p<0.0001$) and AGE scores ($p<0.05$) compared to the NGM group.

The self-assessed physical health of the T2DM group was significantly worse than the NGM group ($p < 0.01$), with 47 % of T2DM participants reporting "poor" or "fair" health, compared to 30% in the NGM group. Both groups reported similar vision status, with 43% of participants reporting their vision status as "good", "very good", and "excellent". All participants received the lutein- and DHA-enriched eggs for 6 weeks and were analyzed for the primary outcomes.

The effects of enriched egg consumption on participant characteristics, self-perceived health and vision status are displayed in **Table 3-2**. Enriched eggs had no effect on body composition, vascular health, or self-perceived health and vision status. The T2DM group had a higher AGEs score than NGM at POST assessment; however, this was not found to be statistically significant after adjusting for *post hoc* analysis ($p=0.07$). There were no other significant differences between participant groups for the parameters in **Table 3-2**.

Enriched egg consumption on retina responses

The effects of enriched egg consumption on photopic (light-adapted) retina responses are displayed in **Figure 3-1**. Cone-driven response to light (msec) was delayed in the T2DM group (PRE 27.33 ± 0.29; DURING 27.52 ± 0.29; POST 27.41 ± 0.29) compared to NGM (PRE 25.83 ± 0.29; DURING 26.02 ± 0.29; POST 26.14 ± 0.29) ($p<0.01$), indicating an overall group effect independent of dietary intervention (**Figure 3-1A**). Enriched eggs did not alter implicit time responses in either participant group. Enriched eggs did not affect photopic maximum amplitude (μv) in either participant group, and there was minimal difference (non-significant) between the

NGM (PRE 16.26 ± 1.08; DURING 16.0 ± 1.08; POST 14.97 ± 1.08) and T2DM (PRE 18.35 ± 1.07; DURING 18.38 ± 1.06; POST 16.90 ± 1.07) groups (**Figure 3-1B**).

The effects of enriched egg consumption on scotopic (dark-adapted) retina responses are displayed in **Figure 3-2**. Enriched eggs over 6 weeks elicited a slower response in scotopic a-wave implicit time in the T2DM group (PRE 15.92 ± 0.45; DURING 16.56 ± 0.41; POST 16.92 ± 0.41) (**Figure 3-2A**); however, this alteration was not found to be significantly different after adjusting for *post hoc* analysis. Scotopic b-wave implicit time increased over 6 weeks after enriched egg intervention in the NGM group (PRE 48.34 ± 1.38; DURING 47.49 ± 1.29; POST 50.87 ± 1.29) (**Figure 3-2C**); however, this alteration was not found to be significantly different after adjusting for *post hoc* analysis. There were no differences between groups for a-wave or b-wave implicit times. Scotopic b-wave maximum amplitude (μv) (rod photoreceptor inner cell response) was slightly higher in T2DM (PRE 51.43 ± 3.32; DURING 51.29 ± 3.07; POST 52.05 ± 3.11) compared to NGM (PRE 44.66 ± 3.33; DURING 43.21 ± 3.20; POST 42.98 ± 3.19) (**Figure 3-2D**; $p=0.038$). There was no effect of the dietary intervention on scotopic a-wave or b-wave maximum amplitudes, and no difference between groups for scotopic a-wave maximum amplitude

The effects of enriched egg consumption on OP responses (mid-retinal cells) are displayed in **Figure 3-3.** OP responses were faster (msec) in the T2DM group (PRE 146.0 ± 3.21; DURING 145.15 ± 2.74; POST 144.39 ± 2.74) compared to the NGM group (PRE 151.16 ± 2.98; DURING 148.83 ± 2.91; POST 151.54 ± 2.85), indicating an overall group effect on implicit time independent of dietary intervention (**Figure 3-3A**; $p<0.05$). Enriched eggs did not alter implicit time responses in either participant group. OP maximum amplitude (μv) was slightly increased in the T2DM group (PRE 37.07 ± 3.04; DURING 39.08 ± 2.68; POST 38.15 ±

2.69) compared to NGM group (PRE 33.31 ± 2.85; DURING 32.63 ± 2.81; POST 31.59 ± 2.75) (**Figure 3-3B**), but this difference was not statistically significant within the current sample power (p=0.06). There was no effect of the enriched eggs on OP maximum amplitude in either participant group.

Enriched Egg Consumption on Macular Pigment

The effects of enriched egg consumption on MPOD are displayed in **Figure 3-4**. Within the T2DM group, estimated and absolute MPOD values (PRE 0.45 ± 0.04; DURING 0.41 ± 0.04; POST 0.40 ± 0.04 and PRE 0.44 ± 0.04; DURING 0.38 ± 0.04; POST 0.38 ± 0.04, respectively) were reduced after enriched egg consumption; however, this difference was not found statistically significant. Enriched eggs did not alter MPOD levels in the NGM group (PRE 0.45 ± 0.04; DURING 0.45 ± 0.04; POST 0.43 ± 0.04 and PRE 0.43 ± 0.04; DURING 0.42 ± 0.04; POST 0.42 ± 0.04, respectively).

Enriched Egg Consumption and Carotenoid Status

The effects of enriched egg consumption on skin-based carotenoid status are displayed in **Figure 3-5**. The T2DM group demonstrated lower skin carotenoid scores at PRE (224.90 ± 12.87 and 280.93 ± 12.87) and POST (224.53 ± 12.87 and 290.90 ±12.87) time points compared to NGM ($p<0.05$). Skin carotenoid scores were also lower at the DURING time point (228.80 ± 12.87 and 227.09 ± 12.94) compared to NGM; however, this measurement was not found to be statistically significant after adjusting for *post hoc* analysis. Enriched eggs did not alter skin carotenoid scores for either participant group.

Enriched Egg Consumption on Plasma Fatty Acids

The effects of enriched egg consumption on plasma fatty acid levels are displayed in **Figure 3-6** and **Table 3-3**. As expected, DHA concentrations significantly increased during

enriched egg consumption from PRE to DURING assessments, and this increase was maintained into POST assessments in both NGM and T2DM groups (**Figure 3-6**; $p<0.0001$). There was no difference between the NGM and T2DM groups for DHA concentrations. Enriched egg consumption also increased total n-3 polyunsaturated fatty acids (PUFA) in both NGM and T2DM groups (**Table 3-3**; $p<0.0001$), with no difference in n-3 fatty acid concentration between groups. Total MUFA concentrations decreased from PRE to DURING assessments in the NGM group ($p<0.05$), and this decrease was maintained into POST assessment. Enriched egg consumption for 6 weeks did not affect total SFA, MUFA, n-6 PUFA, or ALA fatty acids.

Enriched Egg Consumption on Clinical Metabolic Parameters

The effects of enriched egg consumption on clinical metabolic parameters are displayed in **Table 3-4**. Following 6 weeks of enriched egg consumption, there were no significant alterations in glucose, triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol, or C-reactive protein levels in either participant group.

As expected, plasma glucose levels were higher in the T2DM group compared to the NGM group ($p<0.0001$), and this difference was maintained through all three time points (**Table 3-4**). Total cholesterol ($p<0.01$), LDL-cholesterol ($p<0.05$), and HDL-cholesterol levels ($p<0.0001$) were all lower in the T2DM group compared to the NGM group. Total cholesterol and HDL-cholesterol were lower across all three time points, while LDL-cholesterol was only significantly different at the DURING and POST time points. There were no differences between groups for triglycerides and C-reactive protein.

Retina Function and Plasma Profile Correlations

The effects of enriched egg consumption on the relationships between retina function measurements and plasma lipid and clinical metabolic profiles are displayed in **Tables 3-5a-b**.

Table 3-5a displays relationships occurring after 3 weeks of enriched egg consumption (DURING assessments), and Table 5b displays relationships occurring after 6 weeks of enriched egg consumption (POST assessments).

Among the T2DM group after 3 weeks of enriched eggs (DURING assessments), DHA and total n-3 fatty PUFA showed a strong negative correlation with OP maximum amplitude (rs=-0.69 and -0.69, respectively; **Table 3-5a**), glucose was positively correlated with photopic maximum amplitude (rs = 0.42), and CRP was positively correlated with scotopic a-wave maximum amplitude (rs= 0.51). Among the NGM group after 3 weeks of enriched eggs, total SFA were negatively correlated with photopic and OP maximum amplitudes (rs=-0.70 and -0.53, respectively) (**Table 3-5a**).

Only the NGM group experienced correlations for implicit time responses (**Table 3-5a**). Total MUFA, TRIG, and GLUC were positively correlated with photopic implicit time (rs=0.52, 0.61, and 0.45, respectively). LDL-cholesterol was positively correlated (rs=0.56) with scotopic b-wave implicit time responses. Macular pigment (absolute and estimated values) for the T2DM group was positively correlated with TRIG concentrations (rs=0.58 and 0.42, respectively; **Table 3-5a**).

None of the relationships between retina function measurements and plasma lipid and clinical metabolic profiles were maintained after 6 weeks of enriched egg consumption (**Table 3-5b**).

Discussion

The primary aim of the present study was to investigate the effects of consuming lutein- and DHA-enriched eggs on retinal health and metabolic parameters in individuals with T2DM. Following a 6-week intervention, no significant changes were observed in retinal function,

including light-adapted (cone cell) and dark-adapted (rod cell) responses, or macular pigment levels. Additionally, enriched egg consumption had no effects on body composition and plasma lipid profiles.

A secondary aim of this study was to compare the effects of enriched egg consumption between individuals with T2DM and those with NGM. While no significant effects of the enriched eggs were observed between the two groups, there were significant intergroup differences, highlighting the disparity in overall health profiles between individuals with and without T2DM. The consumption of enriched eggs did not affect the body composition and lipid profiles of NGM individuals. Among the metabolic health differences noted, we identified a clear distinction in skin carotenoid levels, as measured by the Veggie Meter®, between the T2DM and the NGM group as this is the first study to identify lower skin carotenoid levels, which reflect plasma carotenoid concentrations (Ermakov et al., 2016) in individuals with T2DM, the role of carotenoid status in the development and progression of T2DM warrants further investigation.

Egg Consumption on Retina Function and Macular Pigment

A previous study demonstrated that the consumption of two lutein-and DHA-enriched eggs daily for 6 weeks improved electrophysiological retina function in healthy older Caucasian adults, a population deemed at risk for age-related macular degeneration (Walchuk et al., 2022). Given the similarities in study design between the current study and that by Walchuk et al. (2022), it was hypothesized that enriched eggs over 6 weeks would also improve electrophysiological retina function in individuals with T2DM, as this population is also at risk for retinal disease (i.e., diabetic retinopathy). The discrepancy between the two studies could be due to the difference in demographics of the study populations. The age range in the Walchuk study was 61-68 years versus 29-80 years in our T2DM group. Since ERG responses are known

to decline with age (Samoto et al., 2020), the response to enriched egg consumption may be more pronounced in older individuals. There was also a difference in sex distribution between our study and that of Walchuk et al. (2022). Our study had almost 50% of each sex in the T2DM group (16m, 14f), while Walchuk's group was predominantly female (11m, 19f). There are sex differences among ERG responses (Ozawa et al., 2014); therefore, within our study, sex differences may be masking the effects of the enriched eggs. A larger sample size may be necessary to detect sex-specific responses to the egg intervention. The present study demonstrated no change in the MPOD levels between the NGM and T2DM groups, while showing a range of 0.38-0.43. Macular pigment values have been shown to peak in subjects in their mid-50s, then significantly decline with age (Castro-Lima et al., 2013).

Additionally, the present study found that two enriched eggs daily for 6 weeks did not affect retina function measures. Previous research has shown that diabetic retinopathy is associated with reduced ERG b-wave amplitude and OP, and these decreases worsen as the retinopathy becomes more proliferative (Luo et al., 2022). In our study, enriched egg consumption was able to maintain ERG photopic and scotopic maximum amplitude levels in the T2DM group, suggesting a potential protective effect against diabetes-related declines in retinal function. In the future, comparing enriched eggs to regular eggs over a longer intervention period, or involving individuals with diagnosed diabetic retinopathy, would be valuable in confirming whether enriched eggs can mitigate retinal function deterioration.

Retina Function in T2DM and NGM

Although the present study did not find significant changes in retinal function due to the enriched eggs, there were significant differences in retinal function between the groups. The T2DM group had significantly slower photopic and oscillatory potential implicit time compared

to the NGM group. This suggests a slower response time for the cells, which may indicate some abnormalities in the outer retinal and amacrine cells responding to the onset of light in the inner retina. These findings are consistent with research that shows abnormalities in oscillatory potential are present in diabetic individuals and animals with and without retinopathy (Li et al., 1992; Shinoda et al., 2007). These findings highlight the fact that individuals with T2DM have damage in their eyes, and the findings of this study are a method to prevent further damage.

A few significant correlations were observed during the enriched egg consumption; however, these were limited to the first three weeks of consumption. Since Walchuk et al. (2022) reported significant effects of the enriched eggs on retina function only after 6 weeks of egg intervention, without measuring the first three weeks of egg consumption, it is possible that in our current study, the retina function correlations occurring at 3-weeks of egg intervention are not due to the enriched eggs, but instead due to background variability or confounding factors within the study population. For example, in the T2DM group, plasma DHA were negatively correlated with OP maximum amplitude, contrary to literature showing positive associations with DHA and ERG amplitudes (Dornstauder et al., 2012; Senapati et al., 2018; Tanito et al., 2009). Additionally, positive correlations were found between plasma glucose and hs-CRP, and ERG amplitudes in the T2DM group, again inconsistent with prior findings (Dmitriev et al., 2024; Lelyte et al., 2022). Given that no significant correlations persisted at 6 weeks, these early findings are likely coincidental and influenced by two confounding factors rather than the egg intervention itself.

Historically, egg consumption has raised concerns for individuals with diabetes due to associations with cholesterol and cardiovascular disease risks (Canadian Diabetes Association, 2023). However, the present study found that consumption of two lutein- and DHA-enriched

eggs daily for 6 weeks did not negatively impact body composition or plasma lipid profiles in individuals with T2DM. These findings align with those reported by Walchuk et al. (2022), which showed that six weeks of daily enriched egg consumption had no adverse effects on lipid profiles in older adults. In contrast, a recent meta-analysis found that whole egg consumption significantly increased body weight and BMI in individuals diagnosed with a chronic disease (Emrani et al., 2023), raising questions about how individuals with T2DM might respond to daily egg consumption. Therefore, the absence of adverse effects on body composition in the current T2DM cohort is encouraging and supports the potential inclusion of eggs as part of a balanced diet for individuals with T2DM (Canadian Diabetes Association, 2019). Future studies with larger sample sizes should consider stratifying T2DM participants by comorbidities such as overweight/obesity and cardiovascular disease to further confirm the safety of enriched egg consumption in populations with more complex health profiles.

Conclusion

The main findings showed that daily consumption of DHA- and lutein-enriched eggs for 6 weeks did not influence retinal function and did not adversely affect blood lipid parameters, body composition, or cardiovascular parameters in individuals with and without T2DM. While apparent differences in health and vision-related measures were observed between the two participant groups, the consumption of enriched eggs itself had no significant effect on these outcomes.

Overall, the findings highlight the distinct health profiles between individuals with and without T2DM and provide evidence that enriched eggs can be safely included in the diet without negative metabolic consequences. These results support existing dietary

recommendations for egg consumption in individuals with T2DM and encourage further research into their potential role in nutrition interventions for diabetes management.

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Author Contributions

CW., JG., and MS., participated in the conceptualization and design of the study; JG. HB, and MS oversaw the study execution; JG. and JC conducted participant recruitment and sample and data collection; JC and CW performed the biochemical analyses; JC and SK conducted the statistical analyses; JC drafted the manuscript; JG and MS reviewed and edited the manuscript. All authors have read and agreed to the published version of this manuscript. MS is responsible to provide overall guidance and financial support for this study.

Table 3-1. Baseline demographics of study participants

Variables	Response	NGM (n=30)	T2DM (n=30)	p-value
Age (years)		55.2 ± 2.4	61.5 ± 2.0	NS
Sex, *n* (%)				
	Male	10 (33.3)	16 (53.3)	NS
	Female	20 (66.7)	14 (46.7)	
Height (cm)		167.9 ± 7.8	170.0 ± 8.7	NS
Weight (kg)		87.1 ± 22.4	92.0 ± 20.0	NS
BMI (kg/m^2)		31.0 ± 1.6	31.6 ± 1.3	NS
	Underweight (<18.5)	0	0	
	Normal (18.5-24.9)	33.3	16.7	
	Overweight (24.9-29.9)	23.3	26.7	
	Obese (>30)	43.3	56.7	
Body Fat (%)		38.3 ± 2.1	38.8 ± 1.7	NS
Systolic BP (mmHg)		133.2 ± 3.8	128.2 ± 2.6	NS
Diastolic BP (mmHg)		83.2 ± 2.0	79.6 ± 1.6	NS
Plasma Glucose (mmol//L)		5.39 ± 0.1	8.96 ± 0.6	<0.01
AGEs		2.3 ± 0.1	2.7 ± 0.1	<0.05
Health Status (%)				<0.01
	Poor	3.0	8.8	
	Fair	26.7	38.2	
	Good	40.0	53.0	
	Very Good	26.7	0.0	
	Excellent	0.0	0.0	
Vision Status (%)				NS
	Poor	16.7	0.0	
	Fair	26.7	44.0	
	Good	36.7	32.0	
	Very Good	3.3	12.0	
	Excellent	3.3	0.0	
Time worrying about eyesight (%)				NS
	None of the time	16.7	23.5	
	Sometimes	66.7	61.8	
	Most of the time	13.3	11.8	
	All of the time	3.3	2.9	

Values are mean ± SEM or percentage for each group. Significant differences between groups were testes by t-test for continuous variables ($p<0.05$) and a Chi-square test ($\chi 2$) for categorical variables.

Abbreviations: BMI, body mass index; AGEs, advanced glycation end products; NS, not significant. NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

Table 3-2. Participant characteristic and self-perceived health and vision during six weeks of enriched egg consumption

	NGM				T2DM				p-value[2]
	PRE	DURING	POST	p-value[1]	PRE	DURING	POST	p-value[1]	
Weight (kg)	87.0 ± 3.9	86.9 ± 3.9	87.1 ± 3.9	NS	91.9 ± 3.9	92.0 ± 3.9	91.4 ± 3.9	NS	NS
BMI (kg/m2)	31.1 ± 1.5	31.2 ± 1.5	31.1 ± 1.5	NS	32.0 ± 1.5	31.9 ± 1.5	31.7 ± 1.5	NS	NS
Body fat (%)	34 ± 3	35 ± 3	35 ± 3	NS	37 ± 3	37 ± 3	36 ± 3	NS	NS
Systolic BP (mmHg)	134 ± 3	132 ± 3	128 ± 3	NS	126 ± 3	126 ± 3	129 ± 3	NS	NS
Diastolic BP (mmHg)	85 ± 2	83 ± 2	83 ± 2	NS	81 ± 2	81 ± 2	82 ± 2	NS	NS
AGEs	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	NS	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	NS	NS
Health status (%)				NS				NS	NS
Excellent	3.7	4.3	4.2		0.0	0.0	0.0		
Very Good	25.9	30.4	29.2		7.4	11.5	8.3		
Good	44.4	39.1	45.8		51.9	46.2	54.2		
Fair	22.2	21.7	16.7		37.0	38.5	33.3		
Poor	3.7	4.3	4.2		3.7	3.8	4.2		
Vision status (%)				NS				NS	NS
Excellent	14.8	13.0	12.5		8.0	8.3	8.3		
Very Good	26.6	17.4	25.0		20.0	12.5	12.5		
Good	33.3	39.1	37.5		44.0	45.8	50.0		
Fair	11.1	17.4	12.5		24.0	12.5	12.5		
Poor	3.7	0.0	0.0		0.0	0.0	4.2		
Time worrying about eyesight (%)				NS				NS	NS
None	92.3	100.0	91.3		96.2	92.0	95.7		
A little	3.8	0.0	8.7		3.8	8.0	4.3		
Some	3.8	0.0	0.0		0.0	0.0	0.0		
Most	0.0	0.0	0.0		0.0	0.0	0.0		
All	0.0	0.0	0.0		0.0	0.0	0.0		

Values are least square mean ± SEM, n=27-29 at PRE, DURING and POST time points per group) or percentage for each group. Significant differences were identified with a mixed-model repeated measures with Turkey-Kramer post hoc test. P-values are significantly different at $p<0.05$ and a Chi-square test ($\chi 2$) for categorical variables.

[1]Test of significance within the group; [2]Test of significance between groups: statistically significant between groups at PRE, DURING and POST, at baseline, WK3 and WK6, respectively.

Abbreviations: BMI, body mass index; AGEs, advanced glycation end products; NS, not significant. NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

Table 3-3. Plasma fatty acid profile of study participants during six weeks of enriched egg intervention

	NGM				T2DM				p-value[2]
%, w/w	PRE	DURING	POST	p-value[1]	PRE	DURING	POST	p-value[1]	
ΣSFA	33.80 ± 0.52	34.11 ± 0.54	34.27 ± 0.52	NS	34.19 ± 0.50	34.21 ± 0.49	34.42 ± 0.50	NS	NS
ΣMUFA	26.18 ± 0.76^{a}	24.49 ± 0.78^{b}	25.13 ± 0.77^{ab}	<0.05	29.79 ± 0.74	29.27 ± 0.73‡	28.83 ± 0.74$^{\#}$	NS	<0.05
Σn-6 PUFA	34.88 ± 0.87	35.71 ± 0.89	35.04 ± 0.87	NS	30.79 ± 0.84	30.92 ± 0.83‡	30.99 ± 0.84$^{\#}$	NS	<0.05
Σn-3 PUFA	3.47 ± 0.14^{b}	4.06 ± 0.14^{a}	4.18 ± 0.14^{a}	<0.0001	3.60 ± 0.14^{b}	4.13 ± 0.13^{a}	3.86 ± 0.113^{ab}	<0.0001	NS
DHA	1.32 ± 0.08^{b}	1.83 ± 0.08^{a}	1.89 ± 0.07^{a}	<0.0001	1.43 ± 0.07^{b}	1.80 ± 0.07^{a}	1.85 ± 0.07^{a}	<0.0001	NS
ALA	0.68 ± 0.05	0.70 ± 0.05	0.78 ± 0.05	NS	0.70 ± 0.05	0.78 ± 0.04	0.71 ± 0.05	NS	NS

Values are least suare means ± SEM (n=26-29 at PRE, DURING and POST time points per group). Data were analyzed using a mixed-model repeated measures with Turkey-Kramer post hoc test. P-values are significantly different at p<0.05.

[1]Test of significance within the group: different superscripts within a row indicate significant differences between PRE, DURING, and POST time points for each group. [2]Test of significance between groups: ‡, # indicate significant differences between groups at DURING and POST time point, respectively.

Abbreviations: ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; Σn-6, total omega-6 fatty acids; Σn-3, total omega-3 fatty acids; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; NGM, normal glucose metabolism; T2DM; type 2 diabetes mellitus.

Table 3-4. Plasma clinical biochemistry profile of study participants during six weeks of enriched egg intervention.

	NGM				T2DM				p-value[2]
mmol/L	PRE	DURING	POST	p-value[1]	PRE	DURING	POST	p-value[1]	
GLC	5.43 ± 0.56	5.48 ± 0.62	5.50 ± 0.65	NS	8.88 ± 3.47*	8.58 ± 2.95‡	9.10 ± 3.70$^{\#}$	NS	<0.0001
TRIG	1.40 ± 0.59	1.36 ± 0.61	1.41 ± 0.58	NS	1.79 ± 1.03	1.87 ± 1.40	1.39 ± 0.56	NS	NS
CHOL	5.02 ± 0.85	5.43 ± 0.86	5.20 ± 0.88	NS	4.19 ± 1.01*	4.29 ± 0.98‡	4.28 ± 1.03$^{\#}$	NS	<0.01
LDL	2.85 ± 0.69	3.25 ± 0.85	3.03 ± 0.78	NS	2.22 ± 0.95	2.35 ± 1.03‡	2.25 ± 1.07$^{\#}$	NS	<0.05
HDL	1.43 ± 0.31	1.44 ± 0.32	1.36 ± 0.28	NS	1.05 ± 0.23*	1.05 ± 0.24‡	1.09 ± 0.22$^{\#}$	NS	<0.0001
CRP (mg/L)	2.40 ± 0.60	2.68 ± 0.62	3.18 ± 0.60	NS	3.85 ± 0.60	2.86 ± 0.60	3.76 ± 0.60	NS	NS

Values are least suare means ± SEM (n=26-29 at PRE, DURING and POST time points per group). Data were analyzed using a mixed-model repeated measures with Turkey-Kramer post hoc test. P-values are significantly different at $p<0.05$.

[1]Test of significance within the group: different superscripts within a row indicate significant differences between PRE, DURING, and POST time points for each group. [2]Test of significance between groups:*, ‡ and #, indicate significant differences between groups at PRE, DURING, POST time point, respectively.

Abbreviations: NGM, normal glucose metabolism; NS, non-significant; T2DM, type 2 diabetes mellitus.

Table 3-5a. Correlation between DURING retinas function measures and DURING plasma lipid and clinical biochemistry profiles

	Group	DHA	Σn-3 PUFA	Σn-6 PUFA	ΣMUFA	ΣSFA	CHOL	LDL-C	HDL-C	TRIG	GLC	CRP
Implicit time												
Photopic	NGM	-0.4079	-0.1683	-0.1993	**0.5235**	0.0805	-0.1572	-0.1729	-0.2527	**0.6134**	**0.4528**	0.0790
	T2DM	0.0513	0.0441	-0.1197	-0.0988	0.0824	-0.1653	-0.0853	-0.3175	-0.0692	-0.2132	0.1575
Scotopic a-wave	NGM	0.0049	-0.1643	-0.1361	0.3716	0.0270	0.0167	-0.0079	-0.0430	0.2879	0.3370	0.0711
	T2DM	-0.1662	0.0458	-0.0162	-0.0439	-0.1504	-0.1063	-0.0657	-0.2436	-0.0424	-0.1427	-0.1755
Scotopic b-wave	NGM	-0.0098	-0.2500	-0.0711	0.1397	0.2083	-0.1158	-0.0509	-0.1351	0.2561	0.3561	-0.1263
	T2DM	0.1031	0.1292	-0.1227	-0.0446	0.2654	0.3227	0.2175	-0.2861	0.2576	-0.2275	0.3633
Oscillatory potential	NGM	-0.0613	-0.0662	-0.0956	-0.0833	0.1789	0.4923	0.5597	-0.0772	0.1088	0.3368	0.2018
	T2DM	0.1130	0.0354	-0.0722	0.0617	0.2061	0.2038	0.0446	0.2400	0.1416	0.0910	-0.3061
Maximum amplitude												
Photopic	NGM	-0.2983	-0.0671	0.4283	0.3044	**-0.7007**	0.1220	-0.0123	-0.0684	0.1175	-0.0842	-0.2649
	T2DM	-0.2280	-0.1856	-0.1347	0.1337	-0.0044	0.0687	-0.0704	0.2183	-0.3409	**0.4172**	-0.3540
Scotopic a-wave	NGM	0.3211	0.0877	-0.1495	0.0319	0.1128	-0.2308	-0.0704	-0.2316	0.0140	-0.0772	0.0246
	T2DM	0.1978	0.0285	-0.2121	0.0467	0.2609	0.1350	0.1097	-0.3752	0.0272	-0.1571	**0.5063**
Scotopic b-wave	NGM	-0.2843	-0.3375	0.0760	0.3701	-0.1667	0.2168	0.0579	0.0228	0.2246	0.2211	-0.0947
	T2DM	-0.0754	-0.0739	-0.0877	0.0262	0.0215	0.2530	0.2006	0.2174	-0.0717	-0.0006	-0.2525
Oscillatory potential	NGM	-0.3225	-0.0932	0.3311	0.1619	**-0.5346**	-0.2353	-0.2238	-0.0904	0.0079	0.0237	-0.2115
	T2DM	**-0.6949**	**-0.6875**	0.0135	0.0244	-0.2027	0.2864	0.3395	-0.1582	-0.1000	-0.2350	0.2864

Values are expressed as Spearman's correlation coefficient (r_s). Correlation is significant at $p<0.05$, bolded for convenience.

Abbreviaations: DHA, docosahexanoic acid; Σn-3 PUFA, total omega-3 polyunsaturated fatty acid; Σn-6 PUFA, total omega-6 polyunsaturated fatty acid; ΣMUFA, total monounsaturated fatty acid; ΣSFA, total saturated fatty acid; CHOL, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TRIG, triglyceride; GLC, glucose; hs-CRP, high-sensitivity C-reactive protein; NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus;

Table 5b. Correlation between POST retinas function measures and POST plasma lipi and clinical biochemistry profiles

	Group	DHA	Σn-3 PUFA	Σn-6 PUFA	ΣMUFA	ΣSFA	CHOL	LDL-C	HDL-C	TRIG	GLUC	hs-CRP
Implicit time												
Photopic	NGM	-0.2023	-0.1745	-0.1798	0.0414	0.2580	0.0257	0.0005	0.2017	0.3550	-0.1162	0.2309
	T2DM	0.1893	0.2310	-0.1566	-0.1288	-0.1501	-0.0170	0.1114	-0.1153	0.0695	-0.1162	0.1884
Scotopic a-wave	NGM	-0.2694	-0.2281	-0.1290	0.1992	0.2219	-0.0714	-0.1494	-0.0020	0.2111	0.3013	-0.0935
	T2DM	0.0481	0.0010	-0.1285	0.0667	0.2901	0.0682	0.0188	-0.1359	0.1169	0.0474	-0.0182
Scotopic b-wave	NGM	0.1022	0.2487	-0.2219	0.2446	0.0712	0.0409	-0.0422	-0.2494	0.2209	0.0403	-0.1806
	T2DM	0.1377	0.3262	0.0628	-0.0100	-0.3163	-0.0850	-0.0712	-0.0386	0.0576	-0.3958	0.1312
Oscillatory potential	NGM	-0.0836	0.0031	-0.3375	0.2859	0.2260	0.1649	0.1273	0.0740	0.0916	0.1766	-0.0130
	T2DM	-0.1468	-0.1808	0.1383	0.0366	0.0810	0.1354	0.0257	-0.0830	0.0943	0.2806	-0.0466
Maximum amplitude												
Photopic	NGM	-0.2406	-0.1880	0.0707	0.2647	0.0256	-0.2411	-0.3488	0.1986	0.0608	0.2362	-0.2204
	T2DM	-0.0717	-0.0948	0.1822	-0.1218	0.1757	0.1118	0.1200	0.3501	0.0333	0.3959	-0.2904
Scotopic a-wave	NGM	0.3581	0.2859	0.0196	-0.1104	-0.3148	0.3078	0.3805	-0.1455	-0.1189	-0.1883	0.1727
	T2DM	0.0175	-0.0761	-0.1048	0.1957	0.0064	-0.0949	-0.1908	-0.1221	0.0452	0.0446	0.0948
Scotopic b-wave	NGM	-0.2714	-0.2694	0.1414	-0.1022	0.2239	-0.0416	-0.0919	0.1104	0.1091	0.4013	-0.0325
	T2DM	0.3130	-0.3923	-0.0385	-0.0820	-0.1196	-0.1136	-0.0919	0.3251	0.2005	-0.1519	-0.0610
Oscillatory potential	NGM	-0.2136	-0.1207	0.3684	-0.1022	-0.1517	-0.3597	-0.3883	0.1234	0.1020	0.2338	-0.1948
	T2DM	-0.0935	-0.0326	0.0593	-0.0090	-0.0553	-0.1176	0.0652	-0.0959	0.1508	-0.0401	0.1085

Values are expressed as Spearman's correlation coefficient (r_s). Correlation is significant at $p<0.05$, bolded for convenience.
Abbreviaations: DHA, docosahexanoic acid; Σn-3 PUFA, total omega-3 polyunsaturated fatty acid; Σn-6 PUFA, total omega-6 polyunsaturated fatty acid; ΣMUFA, total monounsaturated fatty acid; ΣSFA, total saturated fatty acid; CHOL, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TRIG, triglyceride; GLC, glucose; hs-CRP, high-sensitivity C-reactive protein; NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus;

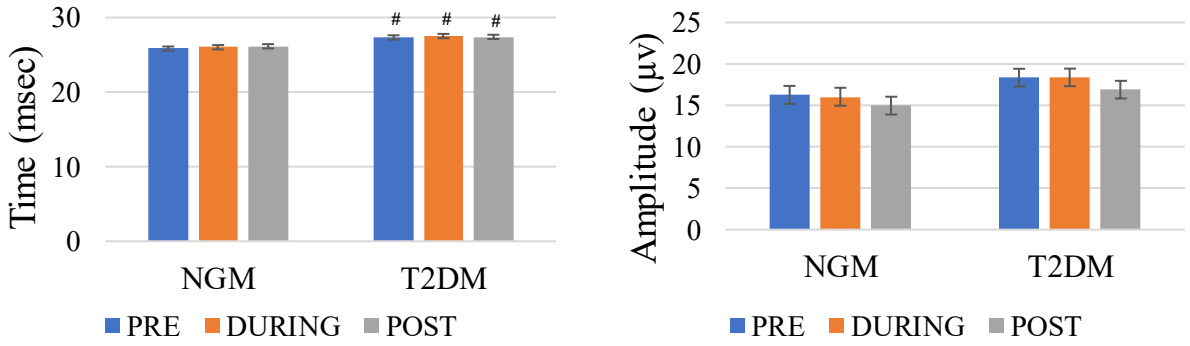

Figure 3-1. Photopic (light-adapted) responses of study participants during six weeks of enriched egg intervention. (A) implicit time (msec); (B) maximum amplitude (µv). Values are least-squares means ± SEM (n= 25-28, an average of both eyes). Data were analyzed using a mixed-model repeated measures with Turkey-Kramer post hoc test. #, significantly different compared to NGM group at the same time point ($p<0.05$).
Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

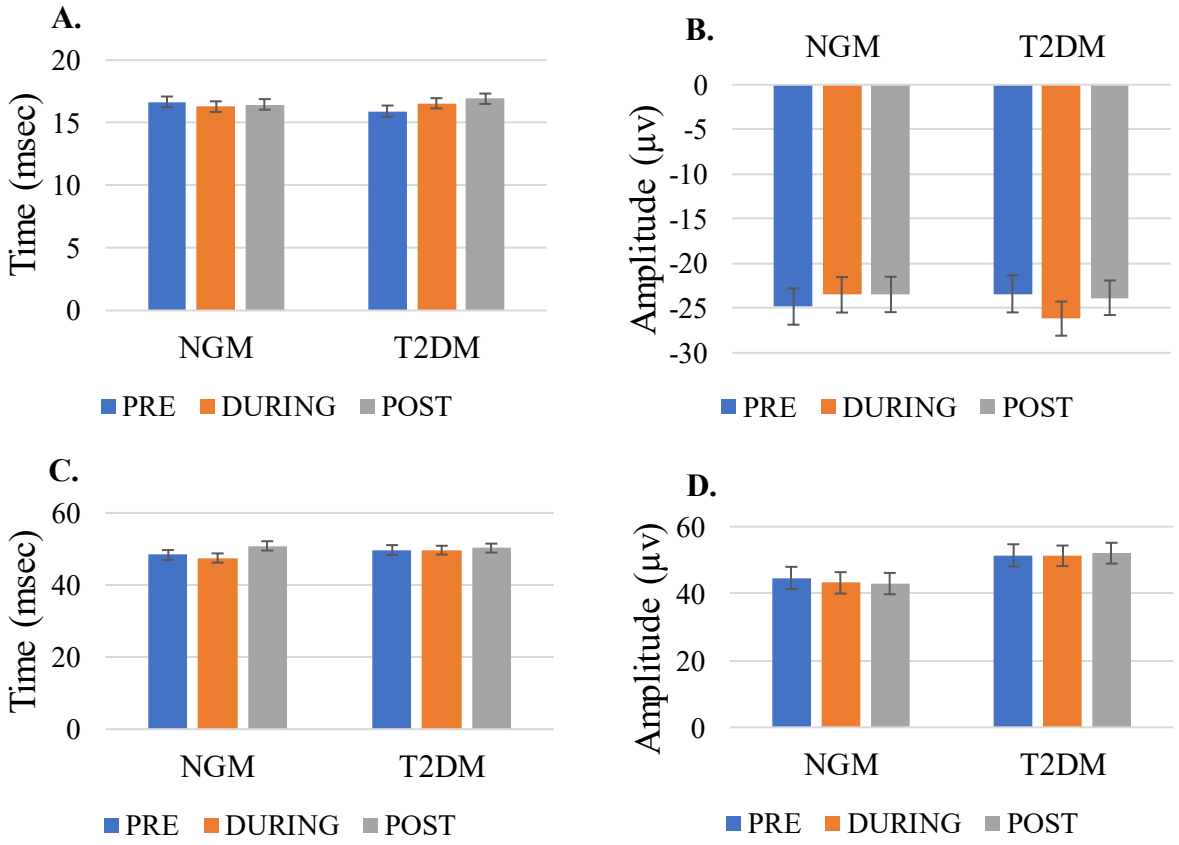

Figure 3-2. Scotopic (dark-adapted) responses of study participants during six weeks of enriched egg intervention. (A) a-wave implicit time (msec); (B) a-wave maximum amplitude (µv); (C) b-wave implicit time (msec); (D) b-wave maximum amplitude (µv). Values are least-squares means ± SEM (n=25-28, an everage of both eyes). Data were analyzed using a mixed-model repeated measures with Turkey-Kramer post hoc test.
Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

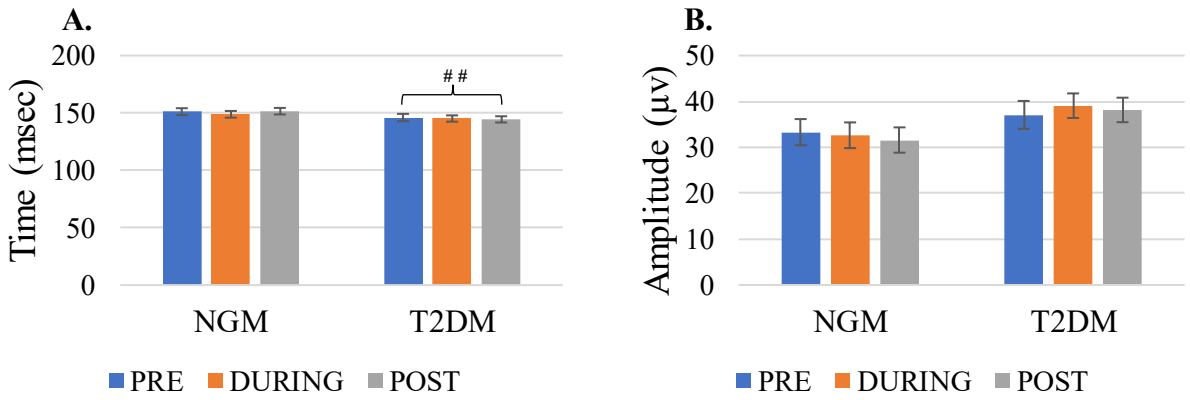

Figure 3-3. Oscillatory potential response (inner retina neuron) responses of study participants during six weeks of enriched egg intervention. (A) implicit time (msec); (B) maximum amplitude (µv). Results are expressed as least-squares means ± SEM (n= 19-26, an average of both eyes). Data were analyzed using a mixed methods repeated measures model with Turkey-Kramer post hoc test. ##, significantl main effect compaed to NGM group ($p<0.05$). Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

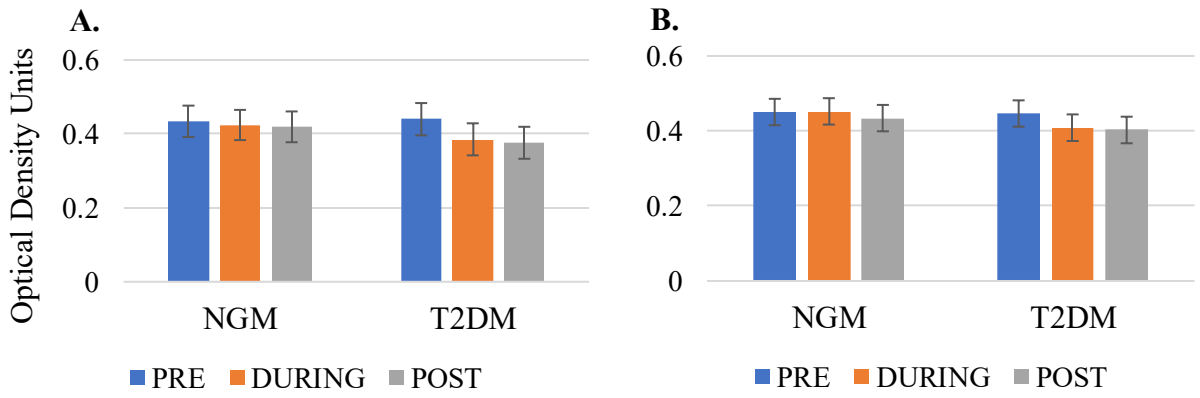

Figure 3-4. Macular pigment optical density of study participants during six weeks of enriched egg intervention. (A) absolute (combined central and peripheral vision) MPOD; (B) estimated (central vision only) MPOD. Results are expressed as least-squares means ± SEM (n=22-30, an average of both eyes). Data were analyzed using a mixed methods repeated measures model with Turkey-Kramer post hoc test.
Abbreviations: NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

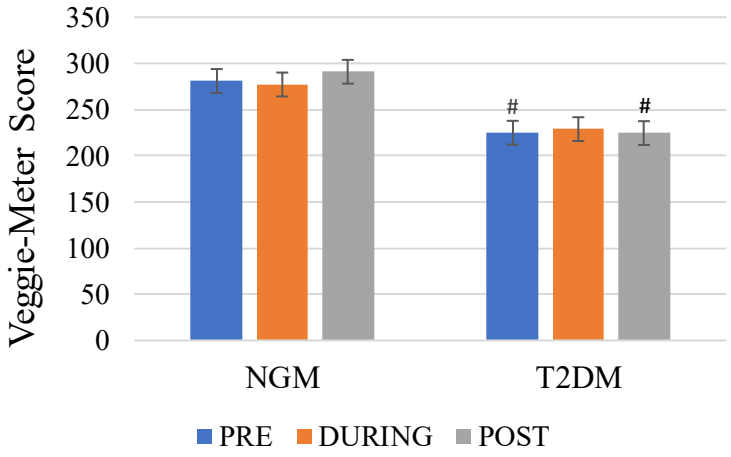

Figure 3-5. Skin-based carotenoid status responses of study participants during six weeks of enriched egg intervention. Results are expressed as least-squares means ± SEM(n = 29-30), Data were analyzed using a mixed methods repeated measures model with Turkey-Kramer post hoc test. # significantly different compared to NGM group within the same time point ($p < 0.05$). Abbreviations : NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

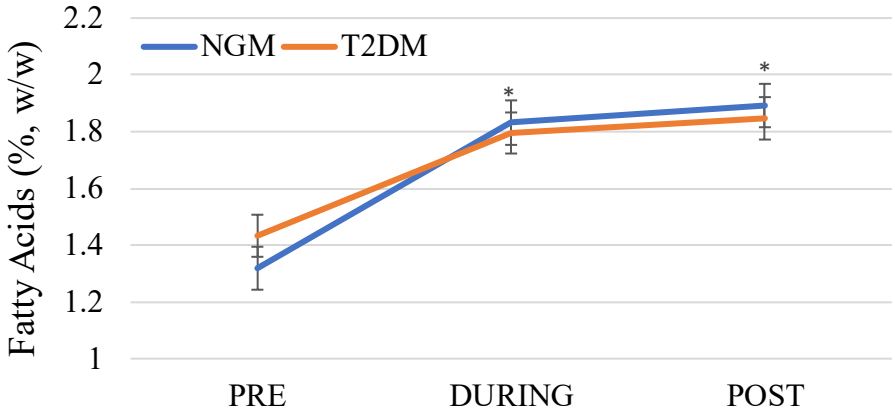

Figure 3-6. Plasma DHA levels responses of study participants during six weeks of enriched egg intervention. Results are expressed as least-squares means ± SEM (n = 21-28). Data were analyzed using a mixed methods repeated measures model with Turkey-Kramer post hoc test. *, significantly different compared to PRE time point within the same group, in both groups ($p < 0.05$).
Abbreviations : NGM, normal glucose metabolism; T2DM, type 2 diabetes mellitus.

Chapter 4. Discussion and Conclusion

The primary aim of this thesis was to assess whether individuals with T2DM without clinically diagnosed diabetic retinopathy exhibit early signs of retinal dysfunction and reduced macular pigment optical density (MPOD), and to evaluate whether these outcomes can be improved through the consumption of DHA- and lutein-enriched eggs, two nutrients known to support retinal and macular health.

Study 1 (Chapter 2) tested the hypothesis that individuals with T2DM have poorer retinal function, lower MPOD, and less favorable metabolic parameters compared to NGM individuals. Despite no significant differences in self-reported vision health, notable differences were observed in objective retinal responses between groups. Individuals with T2DM exhibited significantly delayed photopic and scotopic b-wave implicit times, indicating early retinal cell dysfunction. Lower macular pigment levels were observed in both groups, which is also a concern, as it increases the risk of retinal damage from harmful blue light. These results support the hypothesis that retinal abnormalities exist in individuals with T2DM, even in the absence of perceived vision issues. Thus, the hypothesis regarding retinal dysfunction was supported.

Study 2 (Chapter 3) tested the hypothesis that consuming lutein and DHA-enriched eggs would improve retinal function and MPOD in individuals with T2DM without adversely affecting their plasma metabolic profiles. Following a 6-week intervention, no significant changes were observed in retinal function, MPOD, or metabolic outcomes, including body composition and lipid profiles. Although enriched egg consumption did not significantly improve outcomes, intergroup differences persisted, highlighting underlying health disparities between the T2DM and non-diabetic groups. As a result, the second hypothesis was not supported, possibly due to insufficient dosage or intervention duration.

Strength and Limitations

These studies contain several strengths. One important aspect is that the DHA- and lutein-enriched eggs used in this study are commercially available, making the findings more translatable to real-world dietary strategies. Food and supplements used in clinical trials are often inaccessible to the public, making the results difficult to apply in real-life circumstances. Moreover, the use of a common food, such as eggs, was easy for study participants to consume, as it is already a part of their diet. This increases the likelihood that participants consumed the two medium-sized eggs per day for six weeks, improving compliance. These studies were the first to examine the electrophysiological retinal health and macular pigments of individuals with T2DM and age-matched individuals with normal glucose metabolism in Canada. These results provide an interesting baseline for future studies. Moreover, the comprehensive set of health and nutritional assessments enabled meaningful correlations across various parameters, for example, the novel correlation between skin carotenoid scores and MPOD in individuals with T2DM, which adds to the current literature. The finding also supports the general nutritional safety of egg consumption, supporting no negative impacts on plasma lipids.

While these studies have numerous strengths, they also have some limitations. The absence of a control group consuming regular eggs in study 2 limits the ability to isolate the effects of the enriched nutrients. Due to difficulties in recruitment following COVID, we were unable to obtain enough participants with T2DM to compare DHA and lutein-enriched eggs with regular eggs. This would have allowed for a clear assessment of the DHA and lutein nutrients and their effect on the parameters. Another limitation was the small sample size, which made it impossible to stratify the results, potentially providing a deeper analysis and more significant findings. For example, stratifying the group by the time (in years) of diagnosis could have

elucidated some significant differences between the groups and during the egg trial. Wide variability in diabetes duration, ranging from 6 months to 20 years, meaning the vascular damage resulting from elevated blood glucose concentrations may have affected the outcomes.

Future Research

Further research is warranted to investigate the potential role of lutein and DHA-enriched eggs in preventing or slowing the progression of diabetic retinal changes. In the future, comparing enriched eggs to regular eggs over a longer intervention period, or involving individuals with diagnosed diabetic retinopathy, would be valuable in confirming whether enriched eggs can mitigate retinal function deterioration. A larger sample size would enable us to stratify the T2DM group by other factors, such as gender, comorbidities, and time of diagnosis, which could elucidate some significant differences. Our first study highlighted some significant differences in retinal function and health parameters between individuals with T2DM and NGM. Further studies are needed to determine which retinal cells are abnormal prior to the onset of diabetic retinopathy.

Overall Conclusion

In conclusion, individuals with T2DM in free-living conditions showed evidence of early retinal cell dysfunction, despite no self-perception of vision problems. Moreover, both individuals with T2DM and age-matched individuals with NGM had suboptimal MPOD, which may result in poor protection of the retina. The second study found that 6-week consumption of DHA- and lutein-enriched eggs did not adversely affect retinal function or metabolic health in either group. While no improvement in retinal outcomes was observed, the study adds valuable insights into nutrient-based strategies for retinal health and lays the groundwork for future investigations.

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