Functional Retinal Outcomes and Association with Health Metrics in Patients with Prediabetes and Type 2 Diabetes

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DEDICATION

This thesis is dedicated to all of those who supported and believed in me along this crazy journey, even when I didn't always believe in myself.

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When someone asked me if I would still pursue a Master's degree if I had the choice again, I wholeheartedly said "of course!" Without it, I would never have had the opportunity to get to know Dr. Harrison, who has supported me, mentored me, and pushed me to do things I would never have thought I could do. I am so grateful for you I would also like to thank my co-mentor and committee member, Dr. Richdale, for always talking me through career crises, writing last-minute recommendation letters, and helping guide me through my research journey. A final thank you also needs to be extended to my committee member, Dr. Frishman, for always being on the lookout for opportunities for me to advance in my research career and helping me succeed.

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ABSTRACT

Purpose: Clinical diagnosis of diabetic retinopathy centers around irreversible, damaging vascular changes in the retina. While previous studies have evaluated the neuronal changes that occur in type 2 diabetes (T2DM) before vasculopathy develops, they have not studied glucose dysregulation in prediabetes (preDM) as defined by HbA1c. We aimed to determine if functional metrics are altered in individuals with preDM and T2DM as well as if measures of retinal function in preDM and T2DM are associated with health markers such as anthropometric measures, blood pressure and cholesterol levels.

Methods: The study was carried out in two parts. Subjects were classified by HbA1c measures at the time of testing into control (HbA1c ≤5.6%), prediabetes (5.7-6.4%) and type 2 diabetes groups (previously physician diagnosed or with untreated HbA1c ≥6.5%). In experiment 1, we administered the L'Anthony D-15 color vision test, MARS contrast sensitivity test and recorded mfERG for the right eye and then examined the differences between groups (n = 43 subjects). In experiment 2, we included circumference measures, body fat percentage, blood pressure, and cholesterol levels (n = 34 subjects).

Results: Color vision confusion scores (CVCSs) were significantly different between the three groups (p=0.009) and of all the factors evaluated, had the strongest association with glucose deregulation. There was an association between higher CVCS and higher HbA1c values across groups as well as specifically within the preDM group when controlling for age (R^2=0.29, p=0.01 and R^2=0.39, p=0.02 respectively). Also, hip and waist circumference were associated with HbA1c (R^2= 0.21, p=0.006 and r2= 0.14, p=0.03 respectively). In a multivariate regression,

waist circumference could be used in conjunction with color test results to improve our associative model of HbA1c.

Conclusions: Patients with preDM have functional changes, especially those of color vision, that can be measured in the retina before the diagnosis of diabetes. In addition, tests such as waist circumference and color vision are associated with HbA1c values in patients with preDM and T2DM. These changes could serve as biomarkers that could aid in earlier diagnosis of diabetic eye disease. Further studies are needed to see when these metrics change.

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I. BACKGROUND

This study focuses on alterations in functional retinal outcomes and their association with common health metrics in patients with prediabetes (abbreviated in some chapters as preDM) and types 2 diabetes (T2DM) . In this background chapter, we 1) introduce prediabetes and type 2 diabetes 2) describe ocular complications of diabetes and 3) discuss retinal functional metrics and their association with diabetes in the context of previous literature.

1. Prediabetes and Type 2 Diabetes

Diabetes mellitus is a chronic condition in which the body either cannot produce or effectively use insulin to regulate and metabolize blood sugar, resulting in a hyperglycemic state.[1] This disease affects the world population in epidemic proportions, leading to serious morbidity and mortality.[1, 2] The prevalence of diabetes in the world is on a steep incline, increasing from 180 million individuals in 1980 to 422 million individuals in 2014 encompassing both adults and children.[2] Approximately 1.5 million deaths were caused by diabetes in the year 2012 and the World Health Organization (WHO) predicts that diabetes will move to the 7th leading cause of death by the year 2030.[2]

There are several classifications of the disease, but the most common types are type 1 and type 2 diabetes mellitus.[1, 2] The research reported in this thesis focuses on individuals with type 2 diabetes and prediabetes. Type 2 diabetes is a condition in which body tissue loses its responsiveness to insulin (insulin resistance) and represents 90-95% of cases of diabetes.[1, 2] Although the exact origin of the disease is not clear, the prevalence of diabetes increases in individuals who are overweight and inactive.[2] In order to be diagnosed with diabetes, an

individual must have: 1) a random plasma glucose concentration greater than or equal to 200 mg/dL plus polyuria, polydipsia and weight loss, 2) fasting plasma glucose greater than or equal to 126 mg/dL, 3) a 2-hour plasma glucose greater than or equal to 200 mg/dL during an oral-glucose tolerance test, or 4) HbA1C value greater than or equal to 6.5%.[3]

One group of individuals of special interest that is not included in the values above are those with prediabetes, which affects approximately 34.5% of adults in the United States alone.[4] These individuals do not meet the diagnostic criteria for diabetes but instead have a level of impaired glucose tolerance that leads to an increased risk for developing Type 2 diabetes.[2] Diagnostic criteria for prediabetes includes: 1) a random plasma glucose concentration between 100-125 mg/dL, 2) a 2-hour plasma glucose between 140-199 mg/dL during an oral glucose tolerance test, or 3) and HbA1C value between 5.7-6.4%.[3] Identifying these individuals is key because with proper diet and exercise, regular screening, and medications, their progression to Type 2 diabetes may be delayed or prevented.[2]

2. Ocular Complications of Diabetes

Diabetic retinal disease, which manifests as either diabetic retinopathy or diabetic macular edema, is caused by microvascular compromise in diabetic individuals. [5-7] Within the diabetic population, over one-third of diabetic patients develop diabetic retinopathy and one-third of these individuals have cases that threaten their vision, making diabetic retinopathy the number one cause of visual impairment in adults from ages 20 to 70.[6, 7] Patients who suffer from vision loss due to diabetic retinopathy are predicted to have a higher mortality rate than diabetic patients without retinopathy, particularly from heart disease and stroke.[8]

Complications in the retina are caused mainly by chronic hyperglycemia which leads to pathological changes in the vascular endothelium through various independent mechanisms. These changes, which include the separation of tight junctions in the capillaries and pericyte splitting, ultimately manifest in many observable signs in the back of the eye.[9] Changes that occur in diabetic retinopathy are typically continuous and can become severe. Individuals with non-proliferative diabetic retinopathy (NPDR) may present with micro-aneurysms, dot and/or blot hemorrhages, focal ischemia, and/or venous beading during a fundus examination.[5, 9] These signs present as the patient moves through the stages of NPDR. Individuals may progress to proliferative diabetic retinopathy (PDR) which is the most sight-threatening form as 50% of eyes with this condition will become blind within a period of 5 years.[5] Signs of PDR include new vasculature or fibrous proliferation on or within one disc diameter of the optic disc or anywhere on the retina, preretinal hemorrhage, and/or vitreous hemorrhage.[5,9] Diabetic macular edema may occur at any point during the disease process and should be closely monitored for as it is the main cause of vision impairment during diabetic retinopathy.[5, 9] The severity of the disease mostly depends on how long a patient has had diabetes, but glycemic and cholesterol levels also play a role.[5, 9]

Current treatment options for diabetic retinopathy include pan-retinal photocoagulation (PRP), intravitreal injections of anti-VEGF for diabetic macular edema and/or proliferative disease, and vitrectomy for clearing vitreous hemorrhage, removing fibrous tissue and relieving retinal detachment from vitreous traction.[5, 8, 9] These treatments focus on management as opposed to prevention of the disease.[9] The only strategy for prevention and delaying diabetic retinal disease currently is metabolic control of factors such as blood glucose and blood pressure.[5, 9] Reducing HbA1c levels below seven percent can reduce microvascular complications, though glucose

control should ultimately be individualized based on the age, weight, associated comorbidities, and demographic factors of the patient.[5]

Because diabetic retinopathy is often asymptomatic in initial stages of the disease, it is very important to monitor diabetic individuals for ocular changes to decrease vision loss. [5] Diabetic retinopathy may manifest both in structural and functional examination techniques as it is thought to be both vascular and neurologic in nature.[5, 9] One of the most important structural ocular examination components is a dilated retinal examination along with fundus photography to look for and diagnose lesions or edema (hemorrhages, exudates, neovascularization, etc.) commonly associated with diabetic retinal disease.[5, 6, 10] The use of optical coherence tomography (OCT) is also useful as it provides meaningful information about retinal thickening in situations where edema may not be seen during the dilated examination or about loss of thickness in inner neuro-retinal layers.[5, 6, 10]

3. Retinal Functional Metrics and Association with Diabetes

In terms of functional testing, individuals with diabetes may show reduction in visual acuity and/or refractive error changes that can be related to diabetic changes, such as fluid absorption in the lens.[9] Some other notable testing metrics in diabetic populations include results of color vision, contrast sensitivity, and multifocal electroretinogram (mfERG) tests, which may be capable of detecting changes in the eye earlier than structural testing.[5-7, 9-14]

Color vision testing may serve as a clinically practical test that may be able to detect changes that have occurred in retinal neurons as well as other areas in the visual system with diabetes.[13] Previous studies have shown that color vision defects in primarily blue-yellow but also red-green

discrimination can develop in diabetes and can precede the detectable development of diabetic retinopathy with fundus examination.[5, 6, 9]

Contrast sensitivity, which examines a patient's ability to discern objects from a background, is decreased in diabetic patients both with and without retinopathy due to changes in the inner retinal neurons (especially in the ganglion cell layer), as well as in post-retinal areas.[5, 6, 9-12] This decreased contrast sensitivity has also been shown to occur before retinal changes are detectable, which implies that contrast sensitivity testing could be useful in detecting early diabetic changes in the eye.[5, 6, 9]

Another clinically important test in identifying early diabetic changes in the retina is the multifocal electroretinogram (mfERG), which evaluates local electrophysiologic responses from different areas of the retina to localize damage.[15] The mfERG may be the most sensitive test for diabetic retinal changes because it is dominated by the responses of cone bipolar cells lying close to retinal capillaries, which allows the testing method to rely on both the neural and vascular nature of the retina.[9] Upon mfERG evaluation, individuals with short-term and long-term diagnosed diabetes have been shown to have delayed responses, measured as an increase in implicit time of the positive peak (P1) of the mfERG in both patients with and without vascular retinopathy.[6, 14] In individuals without retinopathy, increased implicit time led to the individuals being more at risk for development of diabetic retinopathy compared to individuals with normal implicit times.[6, 10, 14]

Because of the rising incidence of diabetes as well as its tremendous impact on vision, there should be a focus on diagnosing and treating diabetic eye disease as early as possible to prevent complications. Monitoring and clinical care of diabetic eye disease today largely focuses on the

visible microvascular changes that occur in the eye, sometimes long after damage in the eye has already occurred. Because functional testing such as color vision, contrast sensitivity and mfERG may be useful to identify changes that occur before the development of clinically detectable diabetic retinopathy, it is important to understand their potential function in early identification of changes that could ultimately improve preventative measures in patient care, especially in prediabetic populations.

The objective of the research presented here can be summarized in description of the two studies presented. While previous studies have looked to understand neuronal changes that occur in early type 2 diabetes before vasculopathy has developed, they have not looked at the earliest stages of glucose dysregulation in prediabetes as defined by HbA1c. The objectives of this thesis are to determine if ocular functional metrics are altered in individuals with prediabetes and type 2 diabetes which is presented in Chapter II and to expand this work in Chapter III by evaluating if measures of retinal function in these groups are associated with health markers such as anthropometric measures, blood pressure and cholesterol levels to improve our knowledge on the association between health metrics, ocular functional metrics and HbA1c or diabetes status.

II. FUNCTIONAL RETINAL OUTCOMES IN PATIENTS WITH PREDIABETES AND TYPE 2 DIABETES

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1. Introduction

Diabetes mellitus is an important public health problem. In 2015, diabetes mellitus was estimated to affect 415 million individuals worldwide, with type 2 diabetes (T2DM) accounting for approximately 90% of that value.[16] In spite of increasing knowledge surrounding the disease, global incidence and prevalence of type 2 diabetes is significantly on the rise, with estimates of 642 million individuals being affected by 2040.[16] Chronic hyperglycemia associated with type 2 diabetes causes cellular alterations that leads to damage in the vascular and neurologic systems throughout the body, including the ocular system.[9, 16] Diabetic retinopathy (DR), a complication of T2DM, is one of the leading causes of moderate and severe visual impairment in the world.[9] The number of individuals suffering from blindness from DR has increased by a factor of two from 1990 to 2015.[17] In addition, there are millions of individuals with prediabetes.[16] Prediabetes is a condition in which individuals have elevated blood glucose levels between normal values and the diagnostic criterion for type 2 diabetes, and it is associated with an increased risk of developing type 2 diabetes and related vascular and

neurological complications.[8, 16, 18] However, there is very little data on prediabetes and visual function, likely because prediabetes is not always identified and the effect of prediabetes on vision is poorly understood.

It is well known that retinal functional changes occur in individuals with diabetes mellitus before vasculopathy as evidenced by color vision testing, contrast sensitivity testing and mfERG implicit times.[6, 9-11, 13, 14, 19] Color vision testing may serve as a clinically practical test to identify early changes that have occurred in retinal neurons as well as other areas in the visual system with diabetes.[13, 19] Previous studies have shown that color vision defects in primarily blue-yellow but also red-green discrimination can develop in diabetes and can precede the detectable development of diabetic retinopathy with fundus examination.[6, 9, 19, 20] Contrast sensitivity, which examines a patient's ability to discern objects from a background, is decreased in diabetic patients both with and without retinopathy due to changes in neuronal pathways in the inner retinal neurons (especially the ganglion cell layer), as well as in post-retinal areas, which implies that contrast sensitivity testing could be useful in detecting early diabetic changes in the eye.[6, 11, 12] Lastly, the multifocal electroretinogram (mfERG) evaluates local electrophysiologic responses from different areas of the retina to localize retinal neural damage.[15] Upon mfERG evaluation, individuals with short and long-term diagnosed diabetes have been shown to have an increase in implicit time in patients both with and without vascular retinopathy.[6, 14] In individuals without retinopathy, increased implicit time led to the individuals being more at risk for development of diabetic retinopathy compared to individuals with normal implicit times.[6, 10, 14]

However, it is not known if similar changes in neural function occur in prediabetes, which is one of the earliest stages of glucose dysregulation in the diabetic process. To our knowledge, they have not been included as a separate group in any functional eye study which included all of these tests. The purpose of this study was to evaluate changes in retinal function in patients with prediabetes. Knowing when changes begin in the eye in the course of the disease could be valuable for the development of biomarkers and future treatments.

2. Methods

2.1. Participants

Participants included were between 30 and 70 years of age and were recruited by word of mouth and fliers placed around the University of Houston and University Eye Institute. Participants were classified into one of three groups based on HbA1c measurements at the time of the visit: control (HbA1c $\leq$5.6%), prediabetes (5.7-6.4%) and type 2 diabetes (previously physician diagnosed or with an untreated HbA1c $\geq$6.5%), which corresponds with the ranges published by the American Diabetes Association.[3] Participants who were pregnant females by self-report, had a positive history of eye surgery, had an active eye infection or clinically significant eye inflammation, had known eye disease other than diabetic eye disease, had Type 1 diabetes or were not safe to dilate were not included. Diabetes subjects with retinopathy or retinal edema were not included in this analysis. Participants could not have had known congenital color anomalies. Written informed consent was obtained from all subjects before data collection began. This study was approved by and in compliance with regulations set by the University of Houston's Institutional Review Board and complied with the Declaration of Helsinki. All subjects were consented prior to enrollment.

2.2. Preliminary Testing

All subjects underwent a screening slit lamp biomicroscopy examination using a Haag-Streit Diagnostics BQ 900© instrument (https://www.haag-streit.com/haag-streit-usa/products/haag-streit-diagnostics/slit-lamps/bq-900-led/). Gross examination of the cornea and lens was performed to check that no lens opacities, abnormalities, or cataracts of any type were present. Visual acuity was taken with the subject's habitual correction at distance using an M&S Smart System® (http://www.mstech-eyes.com/products/detail/smart-systemreg-standard) to ensure the subject was 20/25 or better in each eye.

2.3. Color Vision

The L'Anthony desaturated D-15 test (Good-Lite, Elgin, Illinois, USA; https://www.good-lite.com/Details.cfm?ProdID=375) was administered with all the room lights on. The lighting in the room was fluorescent "natural white lighting" with a color rendering index (CRI) rating of 78 CRI and a temperature of 3500K. Lighting in the room was measured with a StellarNet Inc Black Comet spectrophotometer (https://www.stellarnet.us/) using a cosine diffusor pointed upward from the location the test was done. It was found to have two major peaks with the largest at 544.5 nm (1.61 watts/m^2) and the other at 611 nm (1.28 watts/m^2). The overall illuminance on the surface where the test was conducted was measured to be 302 lux. The subjects wore their habitual correction using the common clinical technique for the test. The test was placed on a black tray and subjects performed the test with the right eye. Beginning with the cap labeled "0", subjects were instructed, "Out of the remaining caps, find the color that looks most similar to the last one in the box." The order of the caps was recorded on two-dimensional color space according to the D-15 score sheet template to evaluate for evidence of protan, deutan

and tritan color deficiencies. Total error scores, color vision confusion score, and color angle were calculated using computer programming designed by Torok[21] from the methodology described by Vingrys and King-Smith.[22] In addition, examiners recorded if the patient passed or failed according to the computer program and would pass or fail the color test if administered clinically based on the following criteria: two crossovers of color space, 3 or more single place errors, or 2 or more other larger errors. The subject failed the test if any of these three criteria were met. It was also noted if the subject scored perfectly on the test, as perfect or not perfect, as this is another easy clinical criterion to employ. The direction of the defect was also noted.

2.4. Contrast Sensitivity

Contrast sensitivity was measured monocularly for the right eye using the Mars Letter Contrast Sensitivity Test (Good-Lite, Elgin, Illinois, USA; https://www.good-lite.com/Details.cfm?ProdID=549) at 60 cm under the same overhead lighting conditions as the color vision testing described in detail above and an additional stand light with habitual correction. Subjects were instructed "to read the letters left to right across each line of the chart and guess even if the letters appear faint." The test was complete when the subject missed two consecutive letters. The log contrast sensitivity score was given by the value at the lowest contrast letter identified just before two subsequently incorrectly identified letters, minus scoring correction for previously missed letters as is standard for the test.

2.5. Pupil Dilation and mfERG

The pupil was dilated with 1% tropicamide and 2.5% phenylephrine to at least 6 mm and the left eye was patched. A ground electrode was placed on the subject's left ear after exfoliation. After

the right eye was anaesthetized with 0.5% proparacaine, a Burian-Allen lens (Hansen Ophthalmic Development Laboratory, Coraville, IA, USA; https://www.hansenlab.com/) filled with Celluvisc (Allergan, Madison, NJ, USA; https://www.refreshbrand.com/Products/refresh-celluvisc) was placed on the cornea to record signals. VERIS 6.4.3 by Electrodiagnostic Imaging (EDI) was used to record the mfERG. The stimulus was displayed on a screen and consisted of an array of 103 hexagons where the luminance of each hexagon alternated in a 4-minute pseudo-random binary m-sequence near 100% contrast between white (200 cd/m^2) and black (<5 cd/m^2) at 75 Hz. A central fixation cross was placed in the middle of the stimulus and room illumination approximately matched that of the stimulus screen. The overall recording time was 4 minutes and was divided into eight 30-second segments. Implicit times at P1 and mfERG amplitude measured from trough of N1 to peak of P1 were measured for the entire eye averaged together and for the fovea. The data processing used one iteration of artifact removal and 10% averaging from neighbors from the first order kernel.

2.6. Fundus examination

After the mfERG was completed, a fundus examination including a photograph and optical coherence tomography was performed for both eyes. A Heidelberg Spectralis OCT (Heidelberg Engineering, Franklin, MA, USA; https://business-lounge.heidelbergengineering.com/us/en/products/spectralis/spectralis/) (software version 6.8) was used with an acquisition rate of 40,000 A scans per second. A 7-line volumetric scan over a 30x5 degree area and a 21-line volumetric scan over a 20x20 degree area over the macula were generated to check for diabetic macular edema and to assure the retina was healthy. Using a Topcon TRC-NW400 non-mydriatic retinal camera (Topcon Medical Systems, Oakland, NJ,

USA; http://www.topconmedical.com/products/trcnw400-literature.htm), fundus photographs were taken on both the undilated left eye and dilated right eye over the central 45 degrees. Photographs were assessed for signs of diabetic retinopathy. Patients with any retinopathy or signs of diabetic edema were excluded from analysis.

2.7. Blood Collection and Biochemical Testing

Fingerstick blood was collected for analysis. After the tip of the third or fourth finger was swabbed with an alcohol pad, a 21-gauge 2.5 mm-penetration lancet was used to stick the finger. HbA1C levels were quantified via Siemens DCA HbA1c analyzer (Siemens Medical Solutions USA, Malvern, PA, USA; https://www.siemens-healthineers.com/en-us/diabetes/diabetes/dca-vantage-analyzer#ADD_INFO) and were used to define control (≤5.6%), prediabetes (5.7-6.4%), and diabetes (≥6.5%) groups.

2.8. Statistical Analysis

Kruskal-Wallis tests were used to evaluate differences between groups. If the overall differences were significant, Mann-Whitney U testing was performed post-hoc to detect which groups were different from each other and Bonferroni corrections were used to account for three comparisons between groups (changing the significant p-value to 0.02). Multivariate regression (which controlled for age differences between groups) and Pearson correlations were used to evaluate the relationship of HbA1c and the functional measures.

3. Results

3.1. Participants

Forty-three participants were enrolled in the study: 15 control subjects, 17 with pre-diabetes, and 11 with type 2 diabetes. Subject characteristics are shown in Table 1. The majority of the participants were female. The control group, prediabetes group and type 2 diabetes group were clinically similar in age (middle aged adults). However, the control group was statistically younger than the prediabetes and diabetes groups (p=0.01), thus regression calculations controlled for age in the model. There was no difference in acuity between the three groups. Subjects in the diabetes group had no retinopathy in either eye and HbA1c levels were overall well-controlled in this group (HbA1c ranging from 6.5-9.1%). In the diabetes group based on self-report, 7 patients were on a diabetic medication alone, 1 patient was on insulin alone, 1 patient was on insulin with an additional diabetic medication, and two were not taking any medications.

3.2. Color vision

Color vision confusion score was significantly related to HbA1c across all groups (and after controlling for age) (adjusted R2=0.29, p=0.01) as well as specifically within the prediabetes groups alone (adjusted R2=0.39, p=0.02, Figure 2-1).

Color vision confusion score was also different between the groups (Table 2-1) and post hoc analysis found significant differences between control and diabetes group (p=0.003), but not significant differences between the prediabetes and diabetes groups (p=0.33). The difference between control and prediabetes groups (p=0.029) approached significance but did not quite

meet the threshold after correction for multiple comparisons. Total error scores for the D-15 test showed a similar result (Table 2-1).

In addition, when evaluating clinical results as would be used in an eye exam, the percentage of patients that would fail the color vision test increased between the prediabetes and control patients but this same jump in percentage did not occur between prediabetes and diabetes subjects. There were only 2 patients in the study who had red/green direction crosses, all other defects noted were tritan in direction.

3.3. Relationship of other functional tests, color vision, and HbA1c

There was a negative correlation between color vision confusion score and log contrast sensitivity across groups (R^2= 0.28, p=0.014, Figure 2-2). The contrast sensitivity was different between the three groups (Table 2-1) and post hoc analysis showed the control and prediabetes participants were not significantly different (p=0.22) and neither were the prediabetes and diabetes groups (p=0.032) but the diabetes group had worse contrast sensitivity than the control group (p=0.017).

There was also a weak correlation between increased color vision confusion score and increased whole eye mfERG implicit time across all groups (R^2=0.09, p=0.05). There were no differences between the 3 groups for the mfERG values (Table 2-1).

Backwards multivariate regression of all of the functional tests together and HbA1c, found only color vision remained significant, indicating that the functional examination metrics may provide redundant data with similar changes in prediabetes where color vision may be the strongest indicator early in the process.

Table 2-1. Primary demographic information and functional retinal outcomes in the study population. Values given in counts and median [IQR].

Group	**Control** *(HbA1c ≤ 5.6%)*	**Prediabetes** *(HbA1c 5.7-6.4%)*	**Diabetes** *(HbA1c ≥ 6.5%)*	**p-values**
n	15	17	11	
Biological Sex Male : Female	4 : 11	4 : 13	3 : 8	
Age *(years)*	41 [35-52]	53 [43-61]	56 [53-61]	0.01
HbA1C *(%)*	5.3 [5.0-5.3]	5.8 [5.7-6.0]	7.0 [6.7-7.5]	0.00
Visual acuity *(logMAR)*	0.0 [-0.04-0.02]	0.0 [-0.08-0.04]	-0.04 [-0.08-0.04]	0.8871
Color Vision Confusion Score	1.12 [1.09-1.73]	1.85 [1.23-2.14]	2.03 [1.28-2.63]	0.009
Color Vision Total Error Score	6.8 [6.7-9.7]	10.2 [7.4-13.2]	11.1 [7.5-14.0]	0.0113
Color Vision Fail %				
Clinical designation	26.7%	70.6%	72.7%	
Computer designation	13.3%	64.7%	72.7%	
Color Vision % of Patients with No Errors	20%	12%	0%	
Color Vision Angle	63.5 [61.3-68.2]	66.3 [60.5-75.4]	66.8 [61.5-73.7]	
Contrast Sensitivity *(logCS)*	1.80 [1.71-1.80]	1.72 [1.68-1.8]	1.56 [1.44-1.76]	0.026
mfERG Whole Eye IT *(ms)*	28.5 [27.8-29.2]	28.3 [27.8-28.9]	29.3 [28.4-30.2]	0.31
mfERG Whole Eye Amplitude *(nV/deg2)*	24.7 [21.0-36.3]	24.7 [21.8-30.3]	26.1 [23.0-28.0]	0.87
mfERG Foveal IT *(ms)*	29.7 [28.9-30.7]	29.4 [28.3-30.6]	29.8 [29.2-30.7]	0.32
mfERG Foveal Amplitude *(nV/deg2)*	82.4 [70.6-99.2]	73.3 [64.6-100.4]	88.8 [62.5-95.6]	0.67

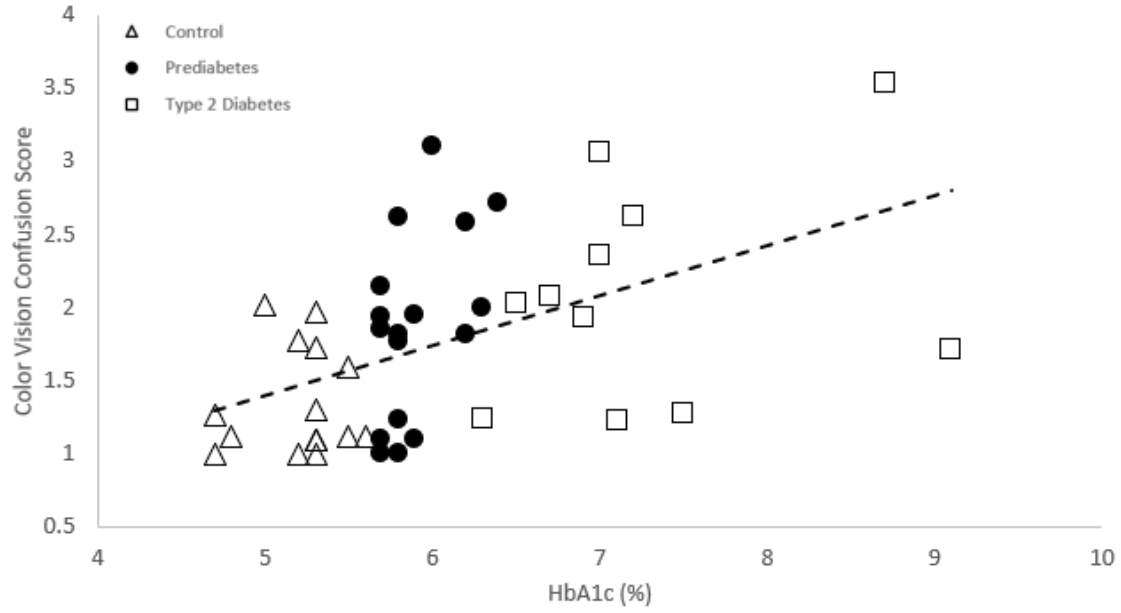


Figure 2-1. HbA1c vs. Color Vision Confusion Score in controls, prediabetes, and type 2 diabetes study groups. (R^2=0.29, p<0.01)

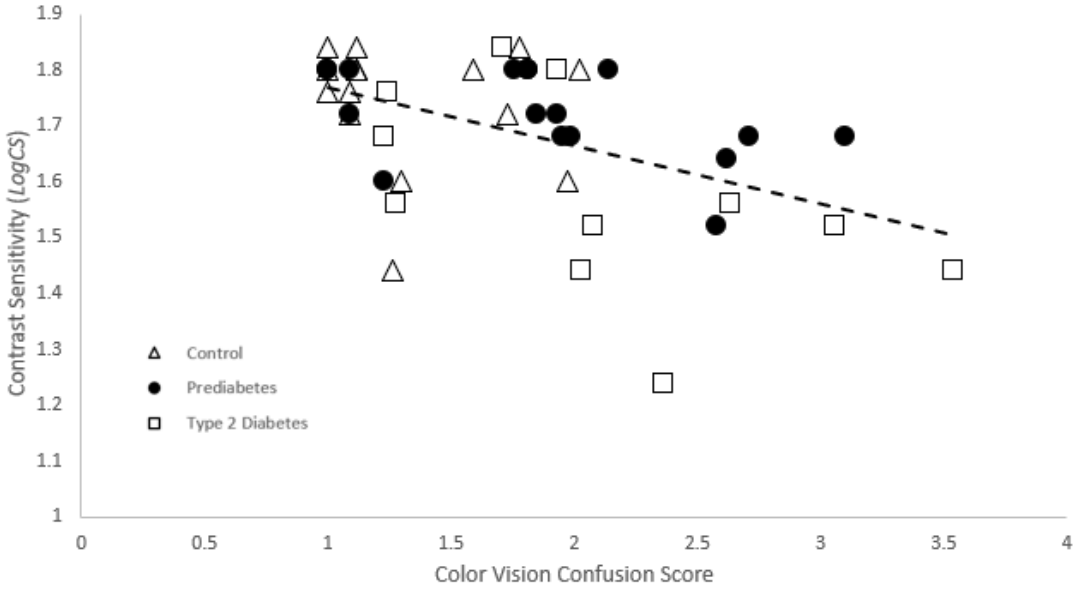


Figure 2-2. Color Vision Confusion Score vs. Contrast Sensitivity in controls, prediabetes, and type 2 diabetes study groups. (R^2= 0.28, p<0.014)

4. Discussion

In this study we found that prediabetes has functional changes that can be measured in the retina before the diagnosis of diabetes. This has important implications for follow up and screening for diabetes within optometric practices. Approximately 5-10% of prediabetic patients convert to type 2 diabetes each year.[23] Thus, prediabetes increases the risk for development of diabetic changes in a significant portion of the population.[23] There have been numerous studies that have looked to understand functional neuronal changes that occur in early type 2 diabetes, including before vasculopathy has developed.[6, 10-14, 19, 20] However, none of these to our knowledge looked at prediabetes as a separate category defined by HbA1c levels at the time of testing.

Similar to other studies, this study found that color vision testing is sensitive to changes caused by diabetes before presentation of clinical retinopathy.[13, 19, 20] However, we were also able to show that alterations in color vision as evidenced by the desaturated D-15 test are associated with HbA1c and can occur as early as the prediabetic stage. Color vision testing in particular appears to have potential to differentiate healthy patients from those with glucose dysregulation as early as the prediabetes stage.

There have been many proposed mechanisms as to how acquired color vision defects occur in diabetes. One study performed in donor retinas found significant reduction in percentage of S-cones within the retina, suggesting this to be the cause of tritan-like deficits in patients with diabetic retinopathy.[24] This coincides with our study, as the subjects in our study almost all demonstrated defects that were tritan in nature. Another proposed mechanism, which seems plausible given our findings in individuals with prediabetes, revolves around the inner retinal circulation. As primarily a vascular disease, damage to the inner retinal microcirculation could

cause alterations in metabolism and fluid changes in the retina that ultimately leads to death and damage of the ganglion cells and cells in the inner nuclear layer.[25, 26] This has been evidenced in other studies, as research performed in a prediabetic rat model has shown thinning in the retinal nerve fiber layer occurring before vasculopathy[18] as well as a study that revealed macular thinning in individuals with prediabetes as diagnosed by fasting blood glucose.[27] There is also the possibility that the changes we are measuring in this study are coming from higher visual centers. Additional testing would be needed to learn if this is true.

Other functional tests such as mfERG and contrast sensitivity have been shown to be significantly altered in individuals with type 2 diabetes before the development of retinopathy in past studies.[10, 13, 14] However, contrast sensitivity and mfERG testing show no significant differences between controls and those with prediabetes in our study. While these tests appear to be redundant and more correlated to each other than to HbA1c, they may still have a place in screening for ocular changes caused by diabetes. More studies are needed with a larger sample and wider ranges of HbA1c to truly understand the relationship between these metrics and screening for changes. Other methods for measuring contrast sensitivity may also need to be evaluated.

One of the limitations of all studies of prediabetes is that we do not know how long subjects have had prediabetes. Other studies have shown that retinal changes in individuals are correlated with duration of diabetes, so examining subjects from the non-diabetic to prediabetic to diabetic state could give us more insight into timing and nature of these changes.[3] Interestingly, the majority of our prediabetes group found out they had HbA1c status consistent with prediabetes during the study and had no knowledge of it before presenting to participate.

In addition, while cataracts or any other visible lens changes were an exclusion criterion in our study, we do not have any objective measure of potential subtle changes in the lens that would not have been visible by slit lamp examination. Lens changes could impact contrast and color testing. Clinically, our groups were very similar by standard subjective testing including acuity, however, the control group was 7-10 years younger than both the prediabetes and diabetes groups. Berninger et al. reported no correlation between age and central color vision thresholds between individuals aged 6 to 71 years after correcting for spectral sensitivity.[28] While our age range was not as large, we did not measure differences in spectral sensitivity in this study. Roy et. al reported no difference in the L'Anthony D-15 test in individuals between the ages of 40 and 55 in normal observers with no lens changes.[29] However, Roy et al. did note color changes in older subjects. While most of our subjects were in the same range as Roy's study, we did include subjects in their 60s. Based on our results and these other studies, we do not believe the lens is responsible for the changes measured but plan to include objective lens assessments in future studies.

Further, although the color confusion and total error scores increase from controls, to prediabetes, to diabetes, it may still be difficult to differentiate these results from each other in a clinical setting based on score or angle alone. The computer program by Torok[21] which uses the methods of Vingrys and King-Smith[22] indicates results similar to the clinical results independently evaluated by the authors. Specifically, it indicated that many of our subjects had "pathological color discrimination, probably diffuse color discrimination error." Longitudinal studies are needed to fully understand the underlying nature of the color discrimination errors seen in this study.

Lighting conditions used for all subjects in the study were similar to typical clinical practice lighting but differed from the ideal lighting for a L'Anthony D-15. We note that bulbs with a bimodal color distribution may not be as useful for color testing as a true light source. The lighting differences in our study may have led to altered perception of the caps. However, it is also important to understand how testing would vary in typical clinical settings, and all subjects here were tested in the same lighting conditions. More work evaluating different lighting would be helpful.

Lastly, we acknowledge that this is a cross sectional study of a small sample of patients with diabetes and prediabetes. The goal was to pilot and explore the most promising screening factors in prediabetes for future studies. A larger sample may show stronger correlations with blood glucose control and other functional measures which were not investigated or significant here.

In the future, we aim to incorporate different lighting and color vision tests such as the anomaloscope, Hardy-Rand-Ritter, or Rabin Cone Contrast Test ,in order to see if these tests produce similar results to the L'Anthony D-15 and to see if one test is more sensitive than the other. We chose the L'Anthony D-15 in this study for its clinical ease of use, however, we acknowledge that it is variable even within the normal population.[30] We also want to follow individuals with prediabetes over time, to see if classical lifestyle interventions are able to reverse the changes that we have observed in color vision.

In conclusion, while functional retinal testing has shown changes that occur in early type 2 diabetes before vasculopathy has developed, there are also functional changes in patients with prediabetes. Color vision testing appears to be a candidate test to differentiate those with glucose dysregulation as early as the prediabetes stage. These color vision errors are positively correlated

with increased HbA1c in patients across a range of prediabetes and diabetes. This study adds to the body of evidence that incorporating color vision testing into diabetes screening protocols may be a helpful tool to detect changes early in these patients. This is important because these changes in color vision could serve as potential biomarkers that may aid in earlier diagnosis of diabetic eye disease and could also provide a measure that could potentially serve as the foundation for earlier treatments.

III. COLOR CONFUSION SCORES COMBINED WITH BODY METRICS ASSOCIATED WITH HBA1C IN PATIENTS WITH PREDIABETES

Portions of this chapter were reported in an abstract and presented as a poster at the American Academy of Optometry's Annual Meeting 2019.

Karson, N, Smith, J, Jones, M, Datta, A, Richdale, K, & Harrison, WW. Color confusion scores combined with body metrics associated with HbA1c in patients with prediabetes. American Academy of Optometry's Annual Meeting; Orlando, FL; 2019. Abstract #195419.

1. Introduction

Prediabetes is a condition in which individuals have a level of impaired glucose tolerance that leads to an increased risk for developing type 2 diabetes.[2] Prediabetes affects approximately 35% of American adults[4] and approximately 5-10% of prediabetic patients convert to type 2 diabetes each year.[23] Thus, prediabetes increases the risk for development of diabetic changes in a significant portion of the population, including diabetic retinopathy and blindness.[23] Many do not know they have prediabetes, so identifying these individuals is key because with proper diet and exercise, regular screening, and medications, their progression to type 2 diabetes may be delayed or prevented.[2]

While primary treatment for this disease is aimed at reducing blood glucose, studies have shown that there is some limit to metabolic control as those with good metabolic control continue to progress and intensive glucose control does not significantly reduce incidence and progression of retinopathy.[31, 32] Because of this, it is important to understand if there are other risk factors that

can contribute to development or progression of diabetic eye disease. Significant factors in the development of type 2 diabetes mellitus along with diabetic retinopathy include visceral obesity, physical inactivity, and other concurrent cardiovascular issues which include hypertension and dyslipidemia.[8] Some studies have shown that development of diabetic retinopathy is associated with visceral fat accumulation or increase anthropometric metrics [33-36] while other studies have shown either no association or even an inverse relationship with the development of diabetic retinopathy. [33, 37, 38]

It is well known that retinal functional changes occur in individuals with diabetes mellitus before vasculopathy as evidenced by color vision testing, contrast sensitivity testing and mfERG implicit times.[6, 9-11, 13, 14, 19] Our group previously reported that reductions in contrast sensitivity and increases in color confusion index are associated with higher HbA1c values in patients with prediabetes and type 2 diabetes mellitus (see Chapter 2).[39]

Due to the discrepancy in evidence as well as lack of studies examining patients with and how ocular metrics correlate with blood measures and anthropometric measurements, we aimed to evaluate if measuring blood and anthropometric measures along with retinal functional metrics (color vision and contrast sensitivity) could add additional information in a model that would strengthen our knowledge about prediabetes and type 2 diabetes in an eyecare setting. Identifying markers associated with prediabetes that can be measured by optometrists in screenings and exams could improve public health through quality referrals and management of diabetic disease.

2. Methods

This study is additive to the study in previous chapter.[39] It is important to note that the data from this experiment was a smaller subset of the same patients collected for the previous chapter. It was also analyzed prior to the experiment in the previous chapter. We included it second as it was a pilot study considering a larger subset of metrics and ultimately helped uncover significant findings to focus on in experiment one.

2.1. Participants

Participants included were between 30 and 70 years of age and were recruited by word of mouth and fliers placed around the University of Houston and University Eye Institute. Participants were classified into one of three groups based on HbA1c measurements at the time of the visit: control (HbA1c $\leq$5.6%), prediabetes (5.7-6.4%) and type 2 diabetes (previously physician diagnosed or with an untreated HbA1c $\geq$6.5%), which corresponds with the ranges published by the American Diabetes Association.[3] Participants were excluded who were pregnant females by self-report, had a positive history of eye surgery, had an active eye infection or clinically significant eye inflammation, had known eye disease other than diabetic eye disease, had Type 1 diabetes or were not safe do dilate. Diabetes subjects with retinopathy or retinal edema were also not included in this analysis. Participants could not have had known congenital color anomalies. This study was approved by and in compliance with regulations set by the University of Houston's Institutional Review Board and complied with the Declaration of Helsinki. Written informed consent was obtained from all subjects before data collection began.

2.2. Preliminary Testing

All subjects underwent a screening slit lamp biomicroscopy examination using a Haag-Streit Diagnostics BQ 900© instrument (Haag-Streit USA, Mason, Ohio, USA). Gross examination of the cornea and lens was performed to check that no lens opacities, abnormalities, or cataracts of any type were present. Visual acuity was taken with the subject's habitual correction at distance using an M&S Smart System® (M&S Technologies, Inc., Niles, Illinois, USA) to ensure the subject was 20/25 or better in each eye.

2.3. Color Vision

The L'Anthony desaturated D-15 test (Good-Lite, Elgin, Illinois, USA) was administered with all the room lights on. The lighting in the room was fluorescent "natural white lighting" with a color rendering index (CRI) rating of 78 CRI and a temperature of 3500K. Lighting in the room was measured with a StellarNet Inc Black Comet spectrophotometer (StellarNet, Inc., Tampa, Florida, USA) using a cosine diffusor pointed upward from the location the test was done. It was found to have two major peaks with the largest at 544.5 nm (1.61 watts/m^2) and the other at 611 nm (1.28 watts/m^2). The overall illuminance on the surface where the test was conducted was measured to be 302 lux. The subjects wore their habitual correction, which is common clinical technique for the test. The test was placed on a black tray and subjects performed the test with the right eye. Beginning with the cap labeled "0", subjects were instructed, "Out of the remaining caps, find the color that looks most similar to the last one in the box." The order of the caps was recorded on two-dimensional color space according to the D-15 score sheet template to evaluate for evidence of protan, deutan and tritan color deficiencies. Total error scores and color vision confusion scores were calculated using computer programming designed by Torok[21] from the methodology described by Vingrys and King-Smith.[22]

2.4. Contrast Sensitivity

Contrast sensitivity was measured monocularly for the right eye using the Mars Letter Contrast Sensitivity Test (Good-Lite, Elgin, Illinois, USA) at 60 cm under the same overhead lighting conditions as the color vision testing described in detail above and an additional stand light with habitual correction. Subjects were instructed "to read the letters left to right across each line of the chart and guess even if the letters appear faint." The test was complete when the subject missed two consecutive letters. The log contrast sensitivity score was given by the value at the lowest contrast letter identified just before two subsequently incorrectly identified letters, minus scoring correction for previously missed letters as is standard for the test.

2.5. Biometric Testing

Biometric data for each subject was recorded. Height (cm) was measured after having the subject remove their shoes using a standard stadiometer. To record body fat percentage and weight (kg), subjects were instructed to wipe the bottom of both feet using the provided alcohol pad and step onto a Tanita Body Composition Analyzer TBF-310 (Tanita Corporation of America, Inc., Arlington Heights, Illinois, USA). For each subject, 0.0 kg was entered for clothing weight, gender was selected based on previous demographic information, standard body type was selected for all patients, and height was entered from previously measured data. Body circumference (cm) was measured to the nearest tenth for the subjects' neck, waist, and hips. Circumference of the neck was measured by placing the superior border of a tape measure below the laryngeal prominence and perpendicular to the long axis of the neck. Waist circumference was measured and the narrowest portion of the waist between the level of the iliac crest and the

lowest palpable rib at a horizontal plane. Hip circumference was measured by placing the tape measure perpendicular to the floor at the largest portion of the buttocks.

2.6. Blood Pressure

Arterial blood pressure was measured for all subjects using an Omron Series 3 BP7100 blood pressure cuff (Omron Healthcare, Inc., Hoffman Estates, Illinois, USA). Subjects were seated with both feet flat on the floor and the cuff was placed on the patient's left upper arm and measurements were taken according to manufacturer's instructions. Results were recorded as systolic blood pressure / diastolic blood pressure in mmHg.

2.7. Fundus examination

A fundus examination including a photograph and optical coherence tomography was performed for both eyes. A Heidelberg Spectralis OCT (Heidelberg Engineering, Franklin, MA, USA) (software version 6.8) was used with an acquisition rate of 40,000 A scans per second. A 7-line volumetric scan over a 30x5 degree area and a 21-line volumetric scan over a 20x20 degree area over the macula were generated to assess macular edema and retinal health. Using a Topcon TRC-NW400 non-mydriatic retinal camera (Topcon Medical Systems, Oakland, NJ, USA), fundus photographs were taken on both the undilated left eye and dilated right eye over the central 45 degrees. Photographs were assessed for signs of diabetic retinopathy. Patients with any retinopathy or signs of diabetic edema were excluded from analysis in this study due to interest in changes before the development of diabetic retinopathy.

2.8. Blood Collection and Biochemical Testing

Fingerstick blood was collected for analysis. After the tip of the third or fourth finger was swabbed with an alcohol pad, a 21-gauge 2.5 mm-penetration lancet was used to stick the finger. HbA1C levels were quantified via Siemens DCA HbA1c analyzer (Siemens Medical Solutions USA, Malvern, PA, USA) and were used to define control (≤5.6%), prediabetes (5.7-6.4%), and diabetes (≥6.5%) groups. Blood was collected via blood glucose testing strips and microcapillary tubes. Blood was analyzed for blood glucose level using a One-Touch UltraMini meter (LifeScan Inc., Milpitas, CA, USA). High density lipoproteins, low density lipoproteins, and total cholesterol levels were tested using an Alere CHOLESTECH LDX 10-959 Analyzer (Abbott Laboratories, Chicago, IL, USA).

2.9. Statistical Analysis

Forward selection multivariate regression (which controlled for age differences between groups) and Pearson correlation was used to evaluate the relationship of HbA1c against the functional measures and health markers. Analysis of variance (ANOVA) testing was conducted to determine if groups were significantly different.

3. Results

3.1. Participants

Thirty-four participants were enrolled in this study: 12 control subjects, 14 prediabetes patients, and 8 with type 2 diabetes. Subject characteristics are shown in Table 3-1. The majority of the participants were female. The control group, prediabetes group and type 2 diabetes group were clinically similar in age (middle aged adults). However, the control subjects were younger (p =

0.02), thus regression calculations controlled for age. In the diabetes group, 7 subjects were on an oral diabetic medication based on self-report.

Table 3-1. Primary demographic information (mean ± standard deviation). * represents significance.

Group	**Control** *(HbA1c ≤ 5.6%)*	**Prediabetes** *(HbA1c 5.7-6.4%)*	**Diabetes** *(HbA1c ≥ 6.5%)*	**p-values**
n	12	14	8	
Biological Sex Male : Female	3 : 9	2 : 12	2 : 6	
Age *(years)*	44.3 ± 9.7	53.6 ± 10.7	54.0 ± 8.6	0.02*
HbA1C *(%)*	5.2 ± 0.3	5.9 ± 0.2	8.1 ± 2.53	<0.001*

3.2. HbA1c vs. Functional and Health Metrics

Color vision, hip circumference, and blood glucose differed by group (Table 3-2). Color vision confusion score had the strongest correlation with HbA1c of any of the vision metrics analyzed (R^2= 0.15, p=0.02, Figure 3-1). When evaluating body metrics, hip and waist circumference were also associated with HbA1c (R^2= 0. 21, p=0.006 and R^2= 0.14, p=0.03 respectively, Figure 3-2). No other factors were significantly associated with HbA1c. In a multivariate regression, waist circumference and color vision confusion score provided the best model to predict HbA1c:

HbA1c = 0.49*Color Confusion Score + 0.019*Waist Circumference + 0.0066*Age

(R^2= 0.31, p=0.02). The other metrics did not add to the multivariate model.

Table 3-2. Mean ± standard deviation for functional retinal and health metrics in control, prediabetes and diabetes groups. * represents significance.

Group	**Control** *(HbA1c ≤ 5.6%) n=12*	**Prediabetes** *(HbA1c 5.7-6.4%) n=14*	**Diabetes** *(HbA1c ≥ 6.5%) n=8*	**p-values**
Color Vision Confusion Score (CVCS)	1.35 ± 0.4	1.77 ± 0.6	1.95 ± 0.7	0.05*
Contrast Sensitivity	1.75 ± 0.1	1.72 ± 0.1	1.65 ± 0.2	0.32
Neck Circumference	35.7 ± 9.0	35.5 ± 4.0	38.6 ± 5.0	0.22
Waist Circumference	89.7 ± 10.7	99.0 ± 16.8	101.7 ± 20.2	0.19
Hip Circumference	106.9 ± 10.1	112.8 ± 11.6	121.0 ± 14.1	0.04*
Weight	77.6 ± 14.4	83.0 ± 19.6	90.9 ± 17.4	0.25
Body Fat %	33.7 ± 9.0	39.6 ± 8.7	39.7 ± 9.2	0.19
High Density Lipoproteins (HDL)	51.4 ± 21.7	58.3 ± 14.8	42.7 ± 15.4	0.17
Low Density Lipoproteins (LDL)	121.0 ± 22.7	119.7 ± 24.2	107.6 ± 19.1	0.45
Total Cholesterol (TC)	211.1 ± 36.2	205.7 ± 21.9	198.7 ± 20.4	0.66
Random Blood Glucose (non-fasted)	109.7 ± 18.8	109.6 ± 12.8	155.4 ± 15.4	0.002*
Systolic Blood Pressure	126.3 ± 15.1	123.6 ± 13.5	130.4 ± 12.5	0.54
Diastolic Blood Pressure	79.7 ± 9.5	81.0 ± 10.3	78.8 ± 12.2	0.88

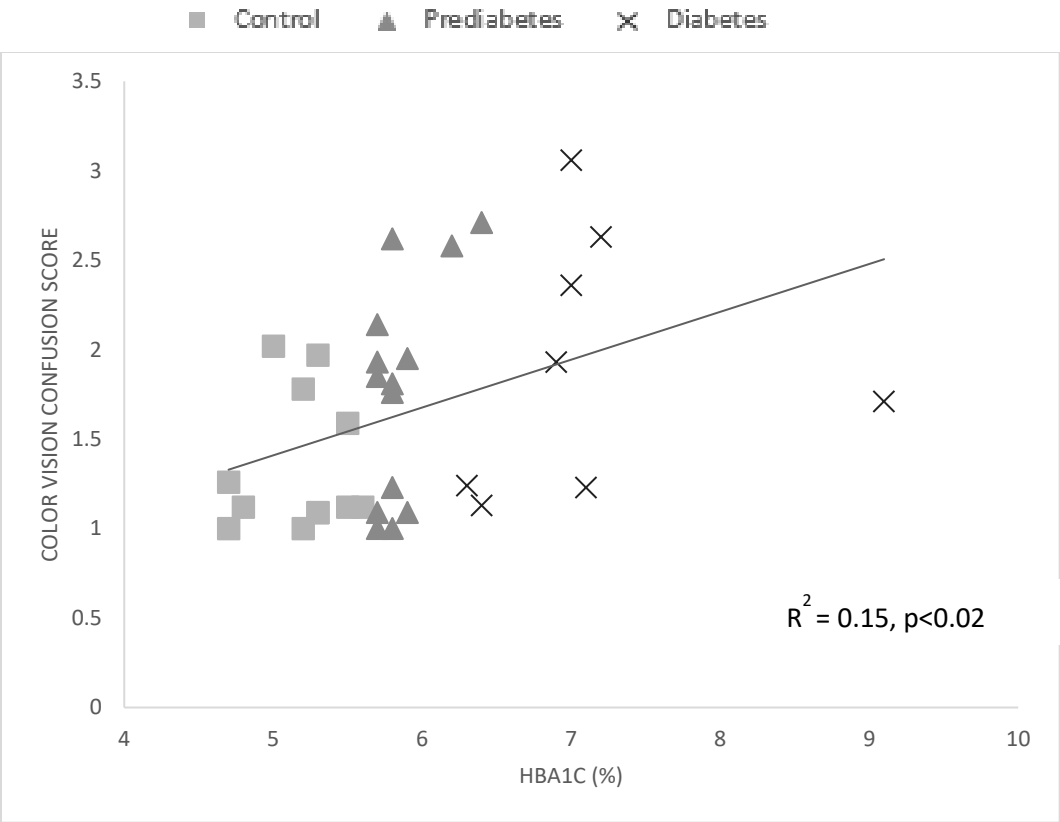
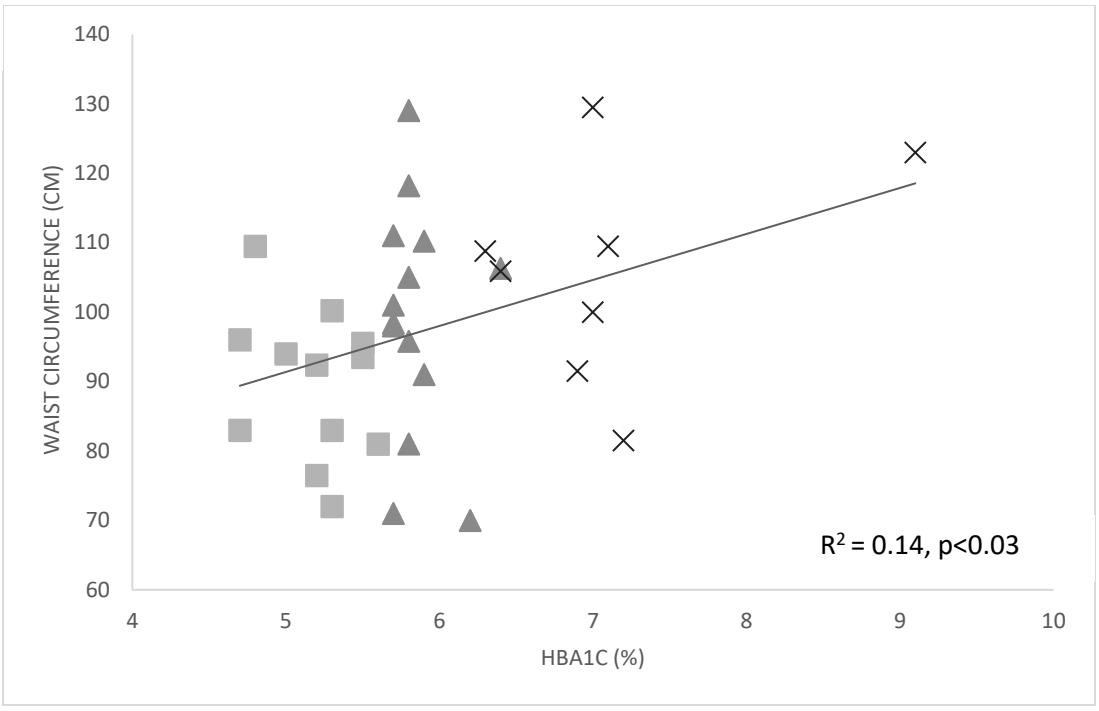


Figure 3-1. HbA1c vs. Color Vision Confusion Score in controls, prediabetes and diabetes study groups.

A)



B)

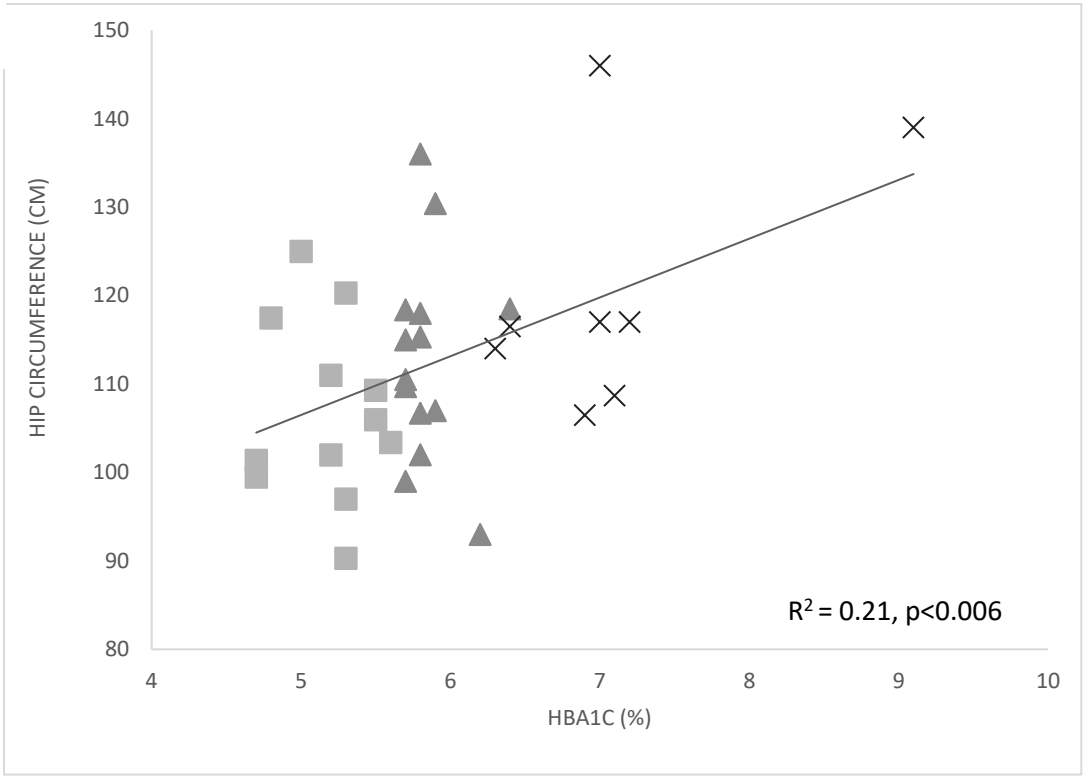


Figure 3-2.
A) HbA1c vs. Waist Circumference in controls, prediabetes and diabetes study groups.
B) HbA1C vs. Hip Circumference in controls, prediabetes and diabetes study groups

4. Discussion

This study found that waist circumference may be an additional additive factor to our previous finding of color vision that may improve our ability to predict HbA1c and diabetes. This is important because these easy to take clinical measures helped to predict HbA1c, which is well documented to be associated with retinal and other diabetes health changes.

Similar to our previous study (Chapter 2)[39], we found that alterations in color vision as evidenced by the desaturated D-15 test are associated with HbA1c and can occur as early as the prediabetic stage.[39] There have been many proposed mechanisms as to how acquired color vision defects occur in diabetes which include significant reduction in percentage of S-cones within the retina,[24] damage to the inner retinal microcirculation that ultimately leads to death and damage of the ganglion cells and cells in the inner nuclear layer,[25-27] and/or the possibility that the changes that we are measuring are coming from higher visual centers which would require additional testing.

We also found that waist and hip circumference were significantly associated with HbA1c, which is consistent with previous research.[40] It is well known that excessive central adiposity is associated with increased metabolic dysfunction and with a higher risk of developing diseases such as diabetes.[8, 41] Common methods of measuring central obesity include anthropometric measurements, body mass index calculations, three-dimensional body scanning, bioelectrical impedance analysis, ultrasound, computed tomography, magnetic resonance imaging and dual-energy x-ray absorptiometry.[41] The benefit of anthropometric measurements is the ease of use, low cost, and non-invasive nature of the testing which leads to their use by organizations such as the International Diabetes Foundation for screening for metabolic disease risk. [41] Tests that could easily be performed as part of an eye screening or exam such as waist circumference and hip circumference are associated with HbA1c values in patients with prediabetes and type 2 diabetes.

This supports use of these tests as easy potential screening options for optometrists if there is a concern about a patient or if working with a high-risk population.

In terms of diabetic eye changes particularly, some studies have shown that development of diabetic retinopathy is associated with visceral fat accumulation [33-36] while other studies have shown either no association or an inverse relationship with diabetic retinopathy. [33, 37, 38] We found that not only were color vision and certain circumference metrics associated with HbA1c, but waist circumference could be used in conjunction with color data to improve our associative model of HbA1c. This could add additional information in a model which strengthens our knowledge about prediabetes and type 2 diabetes in an eyecare setting.

We do acknowledge the limitations of our study including those limitations referenced earlier in Chapter 2, pages 26-28. One limitation includes the study design. As this study was designed as a pilot study to discover the most promising ocular functional metrics and systemic health markers associated with prediabetes, it was a small, cross-sectional study. As diabetic retinopathy is associated with duration of diabetes[5], extending the study into a longitudinal study as well as including more participants could give us more insight into the nature and the timing of the changes that were discovered in this study.

Other limitations include the functional testing that was performed. For example, although lighting conditions were measured and the color vision and contrast sensitivity tests were performed in a clinical setting, we understand that we did not use ideal lighting that was designed for these tests (Illuminant C light source). We do not anticipate that this altered the results as all tests were performed in the same lighting, but we do acknowledge that this serves as a limitation. Future studies should include ideal lighting.

In addition, while cataracts or any other visible lens changes were an exclusion criterion in our study, we do not have any objective measure of subtle changes in the lens that would not have been visible by slit lamp examination. However, all subjects had 20/25 or better acuity and regression analysis controlled for age and thus potential differences in age-related lens clarity, thus the effects of cataracts would be limited.

A final limitation of our study is that the majority of our participants were female. Classifications and guidelines for central obesity are different for males and females based on fat distribution patterns and stature[40, 42]. While waist circumference was significantly associated with HbA1c in our study and contributed to our model, having a separate model for males and females in the future would likely be more clinically applicable. We aim to recruit more subjects, specifically more males to expand our work.

In conclusion, this study showed that color vision errors are positively correlated with increased HbA1c in patients across a range of both prediabetes and diabetes. In addition, body metrics are also correlated with HbA1c and could be used in conjunction with color vision data to predict prediabetes and diabetes. This study adds to the body of evidence that incorporating color vision testing and body metrics into optometric diabetes screening protocols may aid in detecting changes early in these patients. Understanding the relationships that exist between systemic health markers and ocular testing could help us better understand changes that are happening in the diabetic disease process and provide potential screening tools for optometrists if there is a concern about a patient or if working with a high-risk population.

IV. CONCLUSIONS

This thesis examined 1) differences in functional retinal metrics in individuals with prediabetes and type 2 diabetes and 2) relationships between retinal function and health markers such as anthropometric measures, blood pressure and cholesterol levels in prediabetic and diabetic subjects.

The results presented in the thesis have demonstrated:

- Desaturated color vision testing is sensitive to changes caused by diabetes before presentation of clinical retinopathy in patients with type 2 diabetes.
- Alterations in color vision as evidenced by the desaturated D-15 test are associated with HbA1c. Desaturated color vision testing has the potential to differentiate healthy patients from those with glucose dysregulation as early as the prediabetes stage.
- Waist and hip circumference were significantly associated with HbA1c.
- Waist circumference could be used as an overall health marker, in conjunction with desaturated color testing to improve our prediction of diabetes via HbA1c.

This thesis did not address:

- The longitudinal changes that occurred as patients progress from a normoglycemic stat to a prediabetic or diabetic state.
- The influence of biological sex on relationships between the measures of retinal function in individuals and health markers.

It is important to understand if functional changes such as those reported in this thesis and elsewhere occur and are detectable in the prediabetic population because these changes could

serve as potential biomarkers that could aid in earlier diagnosis and serve as metrics for measuring the success of early treatments. In addition, the results of the studies in this thesis could provide screening options for optometrists if there is a concern about a patient or if working with a high-risk population. This also could add additional information to a model that strengthens our knowledge about prediabetes and type 2 diabetes in an eyecare setting and potentially have ramifications in the long run that could help to save sight in these patients.

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