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Chromatic sensitivity loss in subjects at high risk of developing diabetes

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Table of contents

COVID-19 Impact Statement

This statement is provided for the aid and benefit of future readers to summarise the impact of the COVID-19 pandemic on the scope, methodology, and research activity associated with this thesis. The academic standards for a research degree awarded by City, University of London and for which this thesis is submitted remain the same regardless of this context.

Title of the research project: Chromatic sensitivity loss in subjects at high-risk of developing diabetes.

1. Summary of how the research project, scope or methodology has been revised because of COVID-19 restrictions

The scope and methodology were not significantly revised, but the pandemic delayed both the data analysis and the write-up of the thesis. I was supposed to complete my PhD in September 2020, but my completion was delayed until the middle of 2021 so that I could complete my data analysis, write up the thesis, and receive advice from my supervisors. As my supervisors were also dealing with exceptional circumstances, it took them almost a year to begin reading my thesis.

2. Summary of how the research activity and/or data collection was impacted because of COVID-19 restrictions, and how any initially planned activity would have fitted within the thesis narrative

In February 2020, at the start of the pandemic, it was not clear whether students would be able to return to campus. In April 2020, I was sent by my sponsor to Kuwait to work on the frontline of the pandemic, due to my background in clinical medicine. I worked at the Kuwait Airport military base from May 2020 until September 2020. This

necessary hiatus significantly delayed my research during the peak stages of the pandemic.

3. Summary of actions or decisions taken to mitigate for the impact of data collection or research activity that was prevented by COVID-19

The analysis of data was delayed until later in the year. The time taken to write up the thesis was also extended. Once I was no longer working on the frontline of the pandemic, I was able to resume the analysis and write up the findings.

4. Summary of how any planned work might have changed the thesis narrative, including new research questions that have arisen from adjusting the scope of the research project

The eventual study did not diverge too much from the original plan for the thesis.

Date of statement: 01/06/2021

List of Figures

List of Tables

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- Finally: my parents, who have offered their support and spiritual guidance throughout my studies.

Declaration

Dissertation Declaration Form

ID	150050850	Name	QAIS BASTAKI
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I confirm that the work I am submitting for assessment is my own. I declare that the word length of my thesis is 32,218.

I confirm that the electronic copy submitted is an exact copy of the copies submitted for examination.

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Abstract

The primary purpose of this study was to determine if subjects at risk of developing diabetes showed significant loss of colour vision and to identify and grade risk factors for diabetes based on early changes in chromatic sensitivity and rod and cone mediated vision.

Previous studies of diabetic patients without retinopathy had found significant loss of chromatic sensitivity to varying degrees, affecting both Yellow/Blue (YB) and Red/Green (RG) chromatic mechanisms. However, it is unclear whether subjects at risk of developing diabetes also show loss of colour vision, rapid flicker sensitivity and spatial vision when compared to age-matched controls.

For this study, three subject groups were recruited: G1 (the 'normal' subject group, n = 40, who had no risk factors and no history of eye disease); G2 (the 'high-risk' subject group, n = 150, with three or more risk factors for diabetes); and G3 (diagnosed with diabetes, n = 23). RG and YB vision, thresholds for rod- and cone-mediated vision and Visual acuity (VA), functional contrast sensitivity (FCS) were assessed by using Colour assessment and diagnosis (CAD), Flicker sensitivity and Acuity plus tests respectively to assess the overall quality of the spectral, spatial and temporal properties of subject's vision. In addition, each of the risk factors for diabetes was examined in G2 to establish its effects on RG and YB colour thresholds and the loss of rod and cone sensitivity by using multiple linear regression analysis.

The results show that G3 subjects demonstrated the highest loss in colour vision thresholds, rod- and cone-mediated flicker sensitivity and spatial vision when compared to G1 and G2 subjects. Surprisingly, G2 subjects (who did not meet the clinical criteria for diabetes) also had significantly higher RG and YB thresholds and higher rod- and cone-mediated thresholds than G1. Loss of VA and FCS under photopic conditions was also seen. In G2 patients, age above 45 years was a risk factor for RG and YB colour loss. Lack of exercise, age above 45 years and hypertension were risk factors for loss of rod and cone sensitivity.

From this study, loss of both colour vision and rod- and cone-mediated sensitivity are evident and worse in both G2 and G3 subjects when compared to G1. The most significant risk factors in G2 according to this study are advancing age, high blood pressure and lack of exercise. These findings suggest that both RG and YB colour vision thresholds and rod- and cone-mediated rapid flicker sensitivity tests capture best the loss of functional vision in G2 and may therefore be considered an important risk factor in pre-diabetic screening.

List of Abbreviations

ADA – American Diabetes Association

AMD – Age-related macular degeneration

ANOVA – Analysis of Variance

AVOT – Advanced Vision and Optometric Tests

BMI – Body mass index

C – Centre

CAD – Colour Assessment and Diagnosis

CCS – Colour confusion score

CCT – Cambridge Colour Test

CI – Confidence intervals

CINAHL – Cumulative Index to Nursing and Allied Health Literature

EMBASE – Excerpta Medica Database

FCS – Functional contrast sensitivity

FM 100 – Farnsworth–Munsell 100 Hue Test

HbA1c – Glycated haemoglobin

HDL – High-density lipoprotein

IFG – Impaired fasting glucose

IGT – Impaired glucose tolerance

IOP – Intra-Ocular pressure

LL – Lower left

LR – Lower right

OCT – Optical coherence tomography

RCT – Randomised controlled trial

RG – Red/Green

UL – Upper left

UR – Upper right

VA – Visual acuity

YB – Yellow/Blue

1. Introduction

1.1 Overview

This thesis summarizes three years of research into changes in the visual performances of subjects with a high risk of developing diabetes. While it is widely recognised that diabetes is associated with loss of vision and is a leading cause of vision problems in adults, far less is known about the effects of diabetes on colour vision (Lee et al., 2015). Colour vision loss and specific changes or patterns in colour vision impairment may be seen in subjects with diabetes, but little is known about the mechanisms and risk factors for colour vision loss in this population. Furthermore, the timings involved in colour vision loss in subjects in the early stages of diabetes, or those at risk of developing diabetes, has not been accurately established. The potential for colour vision loss and the risk factors associated with it serve as early signs of diabetes. This has significant implications for preventative medicine and targeted approaches to care.

This chapter provides a detailed justification for the research, beginning with an outline of diabetes and an overview of the relationship between diabetes and eye disease. Chapter two reviews the background literature relating to colour vision and visual changes in diabetes. Gaps in the evidence are identified and used to establish the direction for the study, which assessed colour vision in subjects who had been diagnosed with diabetes or were at a high risk of developing it. Chapters three and four give a detailed overview of the methods and the results. In chapters five and six, these results are then considered within the wider context of contemporary research

and clinical practice, with an emphasis on the implications of these findings for the future.

1.2 Definition and epidemiology of diabetes

Type 2 diabetes mellitus (hereafter referred to as ‘diabetes’) is a common condition characterised by peripheral insulin resistance and associated hyperglycaemia. Type 1 diabetes is a condition in which the body cannot produce enough insulin; it is not linked with age or being overweight. In accordance with the general prevalence of type 1 and type 2 diabetes, the vast majority of subjects in this study had type 2 diabetes. A small number had type 1 diabetes. However, as the aim of the study was to assess the visual performance of subjects at risk of developing any form of diabetes, the type of diabetes was not relevant to the results.

In 2019, it was estimated that 463 million individuals around the world are affected by diabetes, though the true number is likely higher, due to variations in the condition’s diagnosis (Saeedi, Pouya (2019). Diabetes is now recognised as a significant public health issue due to the damaging effect it has on so many people’s wellbeing (Qiao et al., 2015). Recently, there have been significant increases in the mortality, morbidity and healthcare spending associated with the disease (Gaskin et al., 2014). Indeed, 15% of all healthcare spending is directly related to diabetes and complications associated with it (American Diabetes Association, 2003; DeFronzo et al., 2015).

The prevalence of diabetes is increasing worldwide. It has increased by approximately 15% in Europe and 30% in the USA. In the Middle East and North

Africa (MENA), however, it has increased by roughly 96% (International Diabetes Federation, 2019). The Middle East, and particularly the Arab states of the Persian Gulf, are thought to have the highest prevalence of diabetes in the world (Alharbi et al., 2014). Given the general prevalence of diabetes in this region, the prevalence in Kuwait, where the current study was conducted, was estimated to be high. Alharbi et al. (2014) found that diabetes was becoming more prevalent in the Gulf states, reaching the level of an epidemic. The mean prevalence of diabetes in the Gulf states is 14.9%, but there is significant variation between the states, and it is estimated that the rate in Kuwaiti adults may be as high as 56% (Al-Kandari et al., 2006; Alharbi et al., 2014). Even within Kuwait, the data can vary based on the population that is examined. For instance, Hasan (2018) recently found that the prevalence of diabetes in a sample of 7,000 oil-sector workers in Kuwait was 15.6%. Meanwhile, Channanath et al. (2014) found that the prevalence was higher among Asian expatriates in Kuwait (33.25%) than among Kuwait natives (25.4%).

1.3 Diabetes pathology

The pathology for diabetes is complex and reflects the accumulated physiological effects of a range of risk factors for the condition (Prasad & Groop, 2015; Murea et al., 2012). The American Diabetes Association (ADA, 2003) has identified several key risk factors for the condition, including a range of modifiable and non-modifiable elements that influence the regulation of blood glucose levels, pancreatic functioning, peripheral insulin resistance and the onset of diabetic complications. The presence of impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) is particularly important for assessing the risk of diabetes. Both of these are objective measures of the insulin response to glucose circulating in the body. Their presence suggests that a

person is pre-diabetic, which means that they are at a high risk of developing clinical diabetes (Tabak et al., 2012). Over time, as these at-risk subjects are exposed to more lifestyle factors associated with diabetes, their risk of becoming diabetic increases.

Diabetes is primarily caused by a loss of insulin sensitivity in the peripheral tissues (leading to insulin resistance) and by pancreatic beta-cell dysfunction (Kahn et al., 2014). Under normal circumstances, glucose levels are maintained through a negative feedback mechanism, in which insulin levels rise in response to circulating glucose, promoting cellular uptake and glucose metabolism. In diabetes, pancreatic dysfunction reduces the level of insulin produced, and the accumulation of peripheral adipose tissue (that is, obesity) reduces the sensitivity of the tissue to the effects of insulin (Nolan et al., 2011). Consequently, physiological insulin responses are no longer sufficient to regulate glucose levels in the blood. Alongside this, inflammatory processes, genetic factors and environmental factors can all contribute to the risk of diabetes.

The primary pathophysiological event in diabetes is an elevation in the amount of glucose circulating in the blood. Over time, elevated blood glucose levels can lead to the glycosylation of proteins and cause damage to blood vessels. Both large vessels and small, peripheral vessels (macrovasculature and microvasculature, respectively) are adversely affected in long-standing diabetes. In the macrovasculature, these changes increase the risk of atherosclerosis and cardiovascular disease. In the microvasculature, these changes can lead to impairment in renal function (nephropathy), nerve function (neuropathy) and vision (retinopathy).

1.4 Diabetic eye disease

As mentioned, the harmful effects of diabetes on the microvasculature extends to the small blood vessels around the eye and the retina. A common manifestation of the disease is diabetic retinopathy, a condition that is associated with visual impairment and is a substantial cause of blindness in adults (Wu et al., 2013). However, diabetes does not only affect the retina; it can also impact multiple aspects of human vision.

Fig. 1 provides a diagram of the human visual system, which is made up of the eye, the optic nerves and the visual processing centres within the brain, known as the visual cortex (Sayin et al., 2015). The eye receives light via a complex process that involves the coordination of numerous structures, such as the cornea, lens and retina (Solomon & Lennie, 2007). Light travels through the cornea, is refracted by the lens and focused onto the retina, which contains a neural layer of photoreceptors. The photoreceptors then convert the light into neural signals that can be transmitted to the optic nerve and the visual cortex.

High-quality vision relies on the structural, functional and physiological well-being of all parts of the eye, optic nerve and brain (Sayin et al., 2015). The refractive capabilities of the cornea and the lens allow light to be focused onto the retina. The integrity of the retina, including the presence and function of photoreceptors, determines the extent to which this light can be transmitted to the brain for interpretation (Rose, 2013). Damage to the retina accounts for much of the sight loss and visual dysfunction associated with diabetes. The remainder of this section will discuss this process in more detail.

According to Fowler (2008), diabetes can manifest in the ocular or visual system in several different ways. After age-related macular degeneration, diabetes is the second most prominent cause of blindness or severe visual impairment in adults (Klein, 2007).

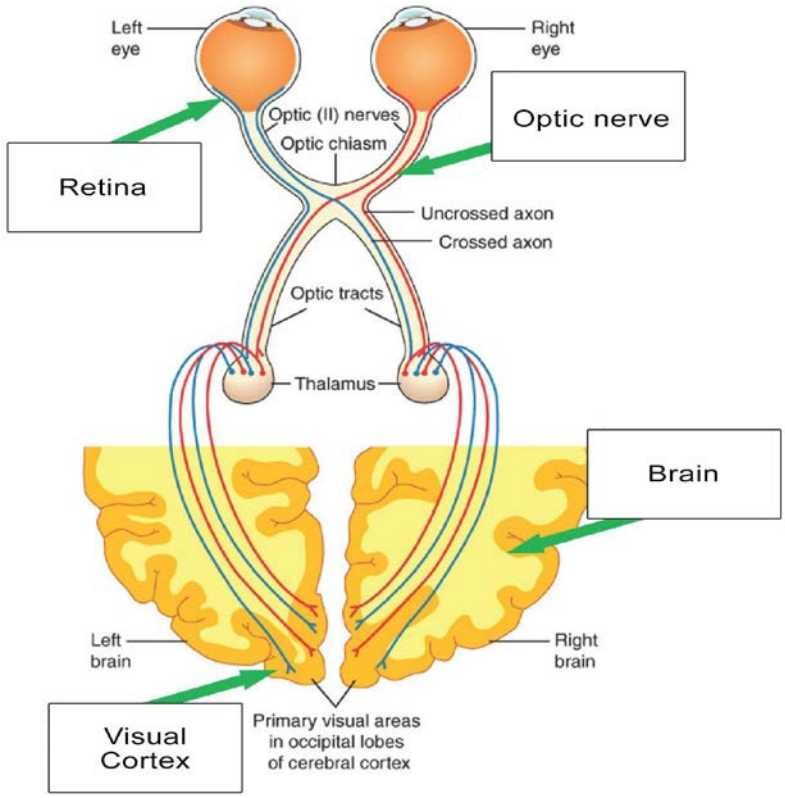

Figure 1: The basic structures of the human visual system (source: F. Robert-Inacio et al., 2012).

The development of diabetic retinopathy is considered one of the most clinically significant manifestations of diabetic eye disease, indicating the onset of severe diabetes (Wu et al., 2013). It is characterised by micro-aneurysms, haemorrhages, ischaemic changes and reduced retinal perfusion, leading to retinal degeneration over time. Although variation in the pathological processes is evident (e.g., proliferative versus non-proliferative disease), outcomes without intervention include macular degeneration, loss of vision and eventual blindness (Fong et al., 2004). Fundoscopy is

the most common method for monitoring retinal changes and tracking the risk of vision loss (Nentwich and Ulbig, 2015).

Diabetic retinopathy is the most widely recognised eye disease associated with diabetes (Bourne et al., 2013). It is commonly found in the diabetic population in Western nations (Hegde et al., 2014; Thomas et al., 2014). In 1984, the Wisconsin Epidemiologic Study of Diabetic Retinopathy found that the prevalence of diabetic retinopathy ranged from 28.8% in individuals who had been diagnosed with diabetes for less than five years to 77.8% in those who had been diagnosed for at least 15 years (Klein et al., 1984a). More recently, it has been estimated that diabetic retinopathy of some form affects 54.6% of adult diabetic subjects over the age of 30 (Hegde et al., 2014). Screening subjects with diabetes across all age groups suggests that the general prevalence of diabetic retinopathy is 30.3%, and 2.9% of diabetic subjects are at risk of losing their sight (Thomas et al., 2014).

In Kuwait, a multi-centre study found that the prevalence of diabetic retinopathy was 43.6% among adults with diabetes who attended primary healthcare centres (Al-Sarraf et al., 2010). Age and length of diagnosis were significant determinants of diabetic retinopathy in this study. Outside this study, there is limited data on diabetic retinopathy in the Kuwaiti population. However, a summary of data from several Gulf states (including Kuwait) suggested a prevalence of 29.9% in subjects diagnosed with diabetes (Omar et al., 2016). These figures are relatively high and suggest that diabetic retinopathy is a common complication of diabetes in the region. These outcomes have a significant impact on patient well-being, daily activities, and mortality, as well as increasing the cost of diabetes care (Hedge et al., 2014).

As the above discussion shows, the link between diabetes and diabetic retinopathy is well established in the research literature. Nevertheless, it is also important to appreciate how vision changes over time and how this may predict the risk of retinopathy in the future. Understanding the preliminary indicators of retinopathy may allow for focused, early treatment or prevention. This is particularly relevant for subjects at risk of diabetes who do not fulfil the criteria for a diagnosis. Hence, screening for retinopathic changes in at-risk populations may be a valuable way of preventing the development of diabetic retinopathy and the onset of its harmful effects.

1.5 Screening for diabetic eye disease

Screening programmes are designed to target at-risk populations or the general population. They must be rigorously assessed to ensure the validity and accuracy of the screening process, the relevance of screening for practice, and the cost-effectiveness of screening (Siu, 2015). The principles underlying effective screening programmes include the need for an accurate test (high sensitivity and specificity), a safe test that is not harmful to patients, and a test that can positively influence patient care (Dobrow et al., 2018). Wilson and Jungner (1968) published ten principles of effective screening, which are considered relevant to current practices (Dobrow et al., 2018). The first principle is that the condition should be an important health problem, which is the case with diabetic eye disease because it affects patient vision and wellbeing. The second principle requires the pathology of the condition to be understood so that a natural history can be ascertained; this is evident in previous studies of diabetic eye disease (Wilson and Jungner, 1968).

Other principles include the potential to identify early markers of disease, the suitability and acceptability of the test, a clear target population, treatment decisions that can be based on the test outcome, a continual process of case-finding, and reasonable costs linked to case-finding (Wilson and Jungner, 1968). It is important to be clear about the optimal population for whom diabetic eye disease treatments may be suited, based on screening patients with pre-diabetes or established diabetes.

Selecting a suitable target population for diabetic eye disease screening is an important consideration. It should be based on risk factor accumulation and the likelihood that specific changes linked to pathology will be detected early. In Kuwait, screening for diabetic eye disease tends to be inconsistent at both the local and the national level. Data from Kuwait suggest that screening and preventative services for diabetic complications are suboptimal, owing to poor awareness of complications among the general population and a lack of clarity in screening guidelines and protocols for healthcare professionals (Al Khalaf et al., 2010; Abdulsalam et al., 2017). Screening processes need to take into account the risk factors in both the general population and those with a strong risk of diabetic retinopathy. High-risk subjects are those who show at least three or more recognised risk factors for diabetes (see Table 2), even though they do not meet the formal diagnostic criteria for the disease (ADA, 2003).

There is a quantitative increase in the risk of developing diabetes that is associated with certain factors. For example, a person who smokes is 1.2–1.4 times more likely to develop diabetes than a non-smoker (Spijkerman et al., 2014). A person with an elevated body mass index (BMI) is four times more likely to develop diabetes than a

person of normal weight (Lotta et al., 2015). Papazafiropoulou et al. (2017) estimated that a person with a parent who has diabetes (mother, father, or both) is between two and four times as likely to develop the disease. People with hypertension are 1.48 times more likely to develop the disease (Centers for Disease Control and Prevention, 2011); those older than 45 are 3 times more likely (Kim et al., 2016); and those with gestational diabetes are 3.5 times more likely (Rayanagoudar et al., 2016). Taken together in various combinations, these risk factors can make pre-diabetic subjects more likely to develop clinical diabetes.

As well as defining the population that should be screened for diabetes, screening programmes need to consider the most appropriate methods for screening. These methods should ensure diagnostic specificity and sensitivity while minimising harm to subjects. Ophthalmological examinations are an integral aspect of screening for diabetic retinopathy, but the identification of specific, objective criteria and pathological events in early or pre-diabetes needs further consideration. This is particularly important as findings from fundoscopy in established diabetes may not provide clear markers for early retinopathy (Fong et al., 2004). The specific use of colour vision assessment to facilitate early identification of disease remains largely untested and confirmation of the accuracy of this technique and the costs associated with this approach are needed to guide optimal implementation in a screening context, as considered in the remaining sections of this chapter.

1.6 Colour vision

Colour vision is a complex phenomenon in humans. It is the result of a long evolutionary history in the development of ocular functioning in vertebrates (Jacobs,

2013). The fundamental building block of colour vision is the cone photoreceptor (Solomon & Lennie, 2007). Photoreceptors within the retina of the human eye are categorised according to their reception of coloured light or black and white light.

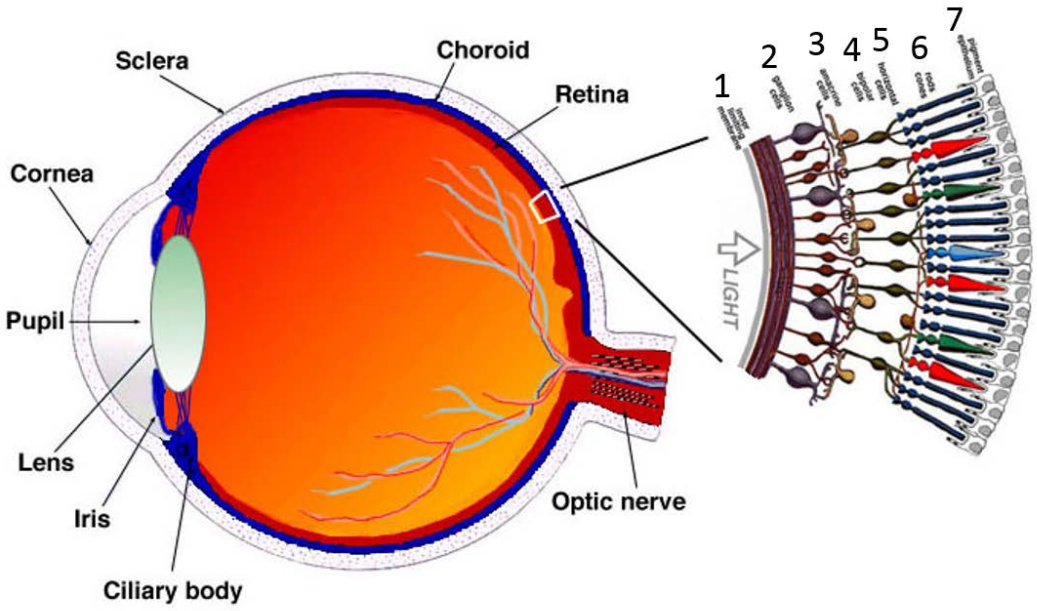

Figure 2: Schematic of a section through the human eye with an enlargement of the retina (adapted from Kolb, 2011).

Note: Layers of the retina are labelled: 1. Inner limiting membrane; 2. Ganglion cells; 3. Amacrine cells; 4. Bipolar cells; 5. Horizontal cells; 6. Rods and cones and 7. Pigment epithelium.

As shown in Fig. 2, when light enters the eye, it passes through ganglion cells and meets the photoreceptor layer of the retina (Solomon & Lennie, 2007). The photoreceptors convert the light signals into electrical stimuli, transmitting this information through bipolar cells to ganglion cells, which form the optic nerve (Euler et al., 2014). Signals then travel along the optic nerve to the lateral geniculate nucleus of the thalamus, where colours are identified and images are understood (Field et al., 2010).

The human eye contains about 130 million rods and about 7 million cones. Rod cells function as specialized neurons that convert visual stimuli in the form of photons (particles of light) into chemical and electrical stimuli that can be processed by the central nervous system. Rod cells are stimulated by light over a wide range of intensities and are responsible for perceiving the size, shape and brightness of visual images (Britannica, 2017). Rod cells have an elongated structure and comprise four distinct regions: an outer segment, an inner segment, the cell body and the synaptic region. The outer segment contains the phototransduction apparatus. It is composed of a series of closely packed membrane disks that contain the photoreceptor molecule rhodopsin. The synaptic region is the site where the rod cell relays its information to the intermediate neurons in the retina. These neurons connect with the ganglion neurons whose axons form the approximately one million fibres of the optic nerve.

Rods are associated with the reception of black and white light, whereas cones are associated with the reception of coloured light. Cones are mostly concentrated within the central retina (the macula), where no rods are present. In contrast, the outer edges of the retina contain fewer cones and many rods. Cones are light-sensitive cells (photoreceptors) with a conical projection in the retina of the eye. As well as being associated with colour vision, they also help with the perception of fine detail. Three classes of cone photoreceptors have been identified. They are arranged in a ‘mosaic’ fashion over the retina with corresponding colour-opponent neurons that compare the signals from each of the cones to allow for the interpretation of colours. These classes are designated as S (short), M (middle) and L (long), depending on the different wavelengths that the cones respond to within the visible spectrum (Solomon & Lennie, 2007; Smith & Pokorny, 1975) (see Fig. 3).

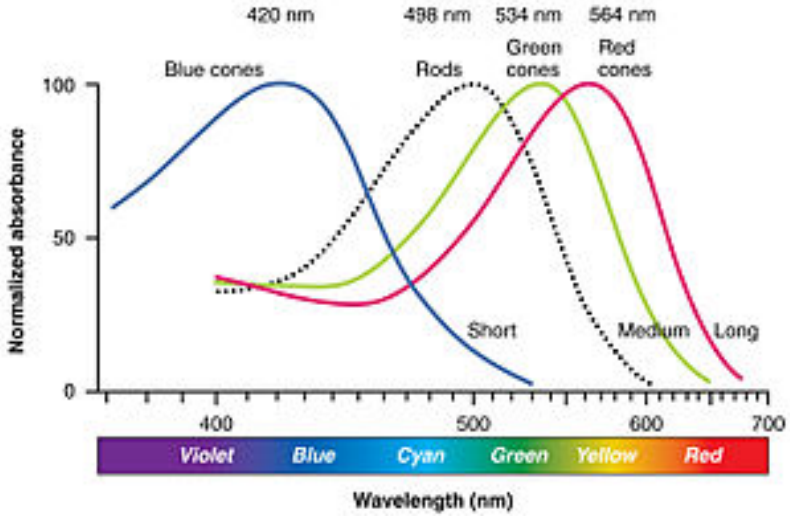

Figure 3: Spectral sensitivities of cone and rod photoreceptors. The x-axis indicates where some colours of the visible spectrum lie (source: Solomon & Lennie, 2007).

A single photoreceptor cannot distinguish between a change in the wavelength of light and a change in intensity. Therefore, clusters of photoreceptors must be compared to provide colour vision (Field et al., 2010). As shown in Fig. 4, opponent processing involves the outputs of L-cones subtracting M-cones to produce the red/green (RG) chromatic channel, while the S-cone signals are subtracted from the sum of L- and M-cones to give the yellow/blue (YB) chromatic channel. The achromatic channel is formed from the sum of 2L + M for the photopic luminance contrast channel and rods for the scotopic luminance channel.

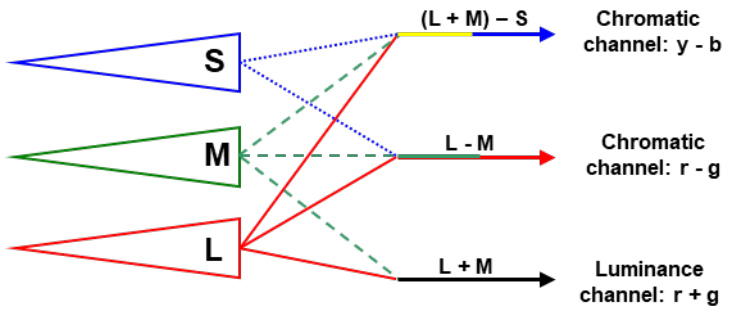

Figure 4: Simple opponent-colour model of normal human colour vision.

Colour vision loss can either be congenital or acquired as a result of pathology. Congenital deficiencies in colour vision arise due to alterations in the genes that encode the molecules responsible for defining the cone photopigment. In this case, the spectral sensitivity of one of the three cone groups can differ significantly from that in normal functioning. It can even be absent or non-functioning. Colour deficiency can be classified according to the number and type of cone photopigments that are present. In most cases, visual acuity (VA) and other visual functions are not affected by congenital colour deficiency, which is binocular, symmetrical, and stable over time. However, this is not true in cases of achromatopsia, where VA is generally poor. There are several different forms of colour loss, which are outlined in detail in the following paragraphs.

Anomalous trichromacy is a condition in which all three types of cone photopigments are present, but one of the three cone photopigments is altered in its spectral sensitivity. The terms protanomaly, deuteranomaly and tritanomaly refer to the presence of abnormal L-, M- and S-cones, respectively. Deuteranomaly is the most common form of anomalous trichromacy, whereas tritanomaly is very rare (see Table 1).

Dichromacy occurs when one class of cone photopigment is missing, and colour vision is reduced to two dimensions (as opposed to the three present in normal trichromats and anomalous trichromats). There are three classes of dichromatism, depending on which of the three-cone pigments is absent. Protanopia, deuteranopia, and tritanopia refer to the loss or absence of L-, M- and S-cone pigments, respectively. The most common forms of dichromatism are deuteranopia and protanopia (see Table 1).

Monochromacy is a form of total colour blindness. Monochromats often claim to perceive colours or associate them with objects by discerning differences in their brightness. It occurs when two or all three of the cone pigments are missing, reducing colour and luminance vision to one dimension (known as complete achromatopsia).

Colour vision loss is more common in men than it is in women. This is because congenital defects are typically inherited as recessive traits found on the X chromosome, which is also the chromosome that determines sex. Congenital colour vision deficiencies affect ~8% of men but only ~0.4% of women. Large surveys have shown that protanopia, protanomalous trichromatism and deuteranopia each occur in approximately 1% of men, and deuteranomalous trichromatism occurs in about 5% of men (Sharpe et al. 1999) (see Table 1).

Table 1: Incidences of colour vision deficiencies in men and women.

Type of Colour Deficiency	Male (%)	Female (%)
Protanopia	1.01	0.02
Protanomalous trichromatism	1.08	0.03
Deutanopia	1.27	0.01
Deuteranomalous trichromatism	4.63	0.36
Number	45.989	30.711
Total prevalence (%)	7.99	0.42

Note: The raw numbers were taken from several different European populations. The table shows that protanopia and deuteranopia are equally frequent in the European male population, while deuteranomaly is four times more frequent than protanomaly. The incidence of tritan defects is very rare. Data obtained from Sharpe et al. (1999).

Tritan loss, which comprises both tritanopia and tritanomaly, is rare in the general population. YB loss affects approximately one in 13,000 people, with men and women affected equally (Post, 1982). Some authors view tritanomaly as a partially expressed form of tritanopia, rather than a separate disorder (Cole et al., 1966; Went & Pronk, 1985). As such, the subtypes of YB loss are often combined as a single category of tritan colour vision defects (Cole, 2007).

Cho and colleagues (2000) have demonstrated that S-cones may be selectively lost in patients with diabetes. The researchers performed terminal deoxynucleotidyl transferase-mediated biotin-deoxyuridine triphosphate nick end labelling (TUNEL) on paraffin sections of five retinas from donors with diabetic retinopathy. S-cones were negative for carbonic anhydrase staining, in contrast to L- and M-cones. The findings suggested that there was a statistically significant reduction in the density of

S-cones in the foveal regions, which was not observed for L- or M-cones ($P < 0.05$). The percentage reduction in S-cones compared with the controls was 21%, a significantly greater reduction than was found in the L- and M-cones. Although this study was based on a small sample size of patients with diabetic retinopathy, there was evidence to suggest that the patients' S-cones were depleted, consistent with the predictors for YB colour vision loss reported in other studies (e.g., Kinnear et al., 1972; Lakowski et al., 1973). Elsewhere in the literature, various defects in rod and cone photoreceptors have also been noted in patients with diabetes and/or diabetic retinopathy (Roy et al., 2017).

However, the pathological mechanisms associated with a loss of chromatic sensitivity in patients with diabetes are only partly understood (Luque et al., 2010). For colour vision loss in general, a wide range of mechanisms have been identified in various disease states; these mechanisms affect chromatic sensitivity, and some of them are likely to apply specifically to patients with diabetes (Cho et al., 2000). Colour vision loss is seen in several ocular, neurologic and systemic disease processes (Simunovic, 2016). Acquired colour vision loss can affect one eye or both eyes (known as unilateral or bilateral disease, respectively). It may be progressive, static, or regressive and may affect only a portion of the visual field (Luque et al., 2010). Five possible mechanisms for colour vision loss in patients with diabetes have been noted in the literature: filtering of shorter wavelengths by optical media (including lens changes); the paucity of S-cones; limited response ranges of S-cones; heterogeneity in the effect of retinal disease; and selective cone fragility (Cho et al., 2000). In practice, there are varying levels of evidence to support the impact of each of these mechanisms. Furthermore, the impact of glucose control on retinal and photoreceptor

function is likely to be a key factor in colour vision loss in the early stages of the diabetic disease, while the relative effects of vasculopathic and neurologic damage to the retina remain unclear. Acquired colour deficiency is often characterised by a diffuse pattern of colour vision loss affecting more than one chromatic channel (Simunovic et al., 2016).

YB colour losses can also be caused by light being filtered out before it reaches the photoreceptors (Cho et al., 2000). Filtering may occur for several reasons, including the presence of cataracts, yellowing of the lens, vitreous or intra-retinal blood, yellowing of the sub-retinal fluid, or the migration of retinal epithelial cells into the retina (Daley et al., 1987). This can affect vision in all three of the colour-confusion axes (i.e., protan, deutan, and tritan), at least during the very early stages of the diabetic disease.

Alterations in the lens of diabetic patients may also be associated with YB colour vision loss (Argirova & Breipohl, 2002). Changes in lens opacity can be observed in patients who have diabetes for a short time, and lens opacity increases as time goes on (Davies & Morland, 2002). Patients with diabetes have a rate of lens yellowing (caused by changes in opacity) that is similar to that in non-diabetic subjects aged 60 years or over (Sparrow et al., 1990; Lutze & Bresnick, 1991). As a consequence, patients with lens opacity changes and lens yellowing may show YB colour vision defects. Indeed, Tregear et al. (1997) found that lens yellowing, rather than defects in S-cone function or morphology, accounted for the YB loss observed in patients with diabetic eye disease. Cataracts have been shown to affect YB chromatic sensitivity in

otherwise healthy patients, even when only moderately developed (Fristrom & Lundh, 2000).

Neuroretinal degeneration is seen as a hallmark of diabetic eye disease in the retina (Du et al., 2013). According to the available literature, retinal neuropathy precedes vasculopathy in patients with diabetes (Adams & Bearse, 2012). Early functional and structural loss of retinal neural tissue is observed during this process, even though the typical symptoms of diabetic retinopathy are not present (Adams & Bearse, 2012). Moreover, the pathogenesis of colour vision loss may relate to wider changes in neuroretinal function and morphology, including the cone photoreceptors and the retinal pigment epithelium (Roy et al., 2017). Therefore, evidence that neuroretinal damage occurs early in the diabetic disease process, with or without vasculopathy, supports the notion that colour vision disruption is an early marker of diabetes.

Abnormalities in rod and cone photoreceptor morphology occur as a natural consequence of ageing, even in the absence of ocular pathology. Individuals who demonstrate these changes with age may be at an increased risk of retinal disease (O'Neill-Biba et al., 2010).

Colour vision testing is a complicated process, and there are several ways of testing for colour vision loss. The Colour Assessment and Diagnosis (CAD) test has been developed by City, University of London in collaboration with the Civil Aviation Authority. It is employed in this study to assess chromatic sensitivity. The CAD test has been employed in several other studies (Seshadri et al., 2005; O'Neill-Biba et al., 2010). Other colour vision tests, and associated visual function testing, are used to

assess rod and cone function and visual acuity, in order to provide a basis for specifically assessing colour vision defects in practice.

The Flicker-Plus test measures flicker modulation thresholds (FMTs) and provides a measure of the temporal responses of the visual system. Specifically, flicker sensitivity testing provides an assessment of temporal contrast sensitivity, which, unlike spatial contrast sensitivity, is affected less by refractive error or scattered light (Bi et al., 2016). This allows for assessment of retinal function in a manner that is largely independent of age-related changes to the eye, or other pathological changes associated with disease processes (Bi et al., 2016).

Assessment of rod and cone function can further be evaluated using the CAD test, which assesses the thresholds of activation of colour vision components according to different axes (yellow-blue and re-green). The normal limits of the CAD test allow for rapid and easy assessment of colour vision loss with age (Barbur & Rodriguez-Carmona, 2015). This test has a high sensitivity and is therefore considered a key test for colour vision assessment and diagnosis of defects (Barbur and Rodriguez-Carmona, 2015). Visual acuity testing is also recommended alongside colour vision-specific tests, as this allows for an insight into age-related or pathology-related changes in the eye, which may otherwise influence interpretation and clinical significance of the results of colour vision testing (Bi et al., 2016). The literature review, provided in Chapter 2, provides further details on colour vision tests.

The literature review, provided in Chapter 2, also considers other colour vision tests. The normal limits of the CAD test allow for rapid and easy assessment of colour vision loss with age (Barbur & Rodriguez-Carmona, 2015).

In summary, the mechanisms that provide colour vision in the human eye are well-established and have been the subject of scientific investigation for several decades, using increasingly sophisticated techniques (Solomon & Lennie, 2007). Identifying disruption in colour vision is of paramount importance in understanding the early signs of diabetic eye disease. The defects of colour vision associated specifically with diabetes are considered in the following section.

1.7 Predictors and risk factors for diabetic retinopathy

Several studies have investigated the early changes associated with retinopathy and the future progression of the disease, but with mixed results. Visual acuity (VA) testing may be an important feature of early eye screening to detect specific changes in visual function, serving as a marker for diabetic retinopathy. Although VA changes may be an early marker of diabetic retinopathy, this finding is inconsistent. It relies on the high-contrast evaluation of spatial resolution, but in many subjects, only their intermediate or low spatial frequencies may be affected (Klein, Klein & Moss, 1984; Nentwich & Ulbig, 2015). Other potential early changes include alterations in adaptation to the dark, contrast sensitivity, glare recovery and colour vision (Gella et al., 2015). Among these, one of the early changes associated with retinopathy in subjects with diabetes is an alteration in colour vision, which has attracted a significant amount of interest among researchers.

Table 2: American Diabetes Association risk factors for diabetes mellitus (adapted from ADA 2003)

ADA risk factors for diabetes
- Previously identified IFG or IGT - Elevated BMI - Habitual physical inactivity - Positive family history - Hypertension (≥ 140/90 mmHg in adults) - Gender (more common in men) - Ethnicity (elevated risk in African Americans, Asian-Americans, etc.) - Age (45 years or over) - Smoking - History of gestational diabetes mellitus or delivery of a baby weighting > 9 lbs - HDL cholesterol ≤ 35 mg/dl (0.90 mmol/l) and/or a triglyceride level of ≥ 250 mg/dl (2.82 mmol/l) - Polycystic ovary syndrome - History of vascular disease

Relatively few studies have recognised the potential value of recognizing colour vision changes as an early sign of diabetes or pre-diabetes (Roy et al., 1984; Daley et al., 1987). As this subject group is a key target for screening, more data are needed to determine if colour vision loss is a characteristic of subjects at risk of diabetes or is associated with future risk of retinopathy, thereby representing a novel screening approach. Gregori et al. (2006) have specifically suggested that this approach should be evaluated further in research settings.

As is typical worldwide, colour vision assessments are not routine for subjects with diabetes in Kuwait. The same is true of the Middle East more generally, though more recently, there has been an increasing awareness of the need to assess colour vision for occupational purposes. This is due to the review of colour vision requirements

carried out by the Civil Aviation Authority (CAA), the EASA, and the Federal Aviation Administration (FAA). This review outlined the minimum colour vision requirements for professional flight crews by assessing the level of colour vision loss above which subjects with colour deficiency no longer performed the most critical, colour-related tasks as accurately as normal trichromats. The CAA recommended that participants must pass the Ishihara colour plate test. Failure to pass this test should prompt a colour vision assessment using anomaloscopy and colour assessment and diagnosis testing (CAA, 2009).

The risk factors for diabetic retinopathy are the same as for diabetes (Table 2). However, diabetic retinopathy generally develops after a clinical diagnosis, once a person has had the disease for longer. Its consequences are significant. Diabetic retinopathy is the leading cause of blindness among adults of working age. Of these individuals, 3-10% will have diabetic macular oedema and 30% will have visual impairment due to diabetic macular oedema and neovascular glaucoma, leading to reduced VA and a reduced visual field (Ting et al., 2016; Liu et al., 2017).

1.8 Aims and hypotheses

The overall aim of this study is to identify individuals at risk of diabetes by evaluating their colour vision at an early stage. This would have implications for preventative strategies in response to diabetes, while also highlighting specific modifiable risk factors that may be linked to the progression of diabetes, particularly when it comes to visual outcomes.

As part of this overall aim, the study has the following objectives:

- To examine the type of colour vision loss in subjects already diagnosed with diabetes, thereby confirming the findings of previous studies.

- To investigate RG and YB thresholds in subjects at a high risk of developing diabetes.
- To assess whether VA, functional contrast sensitivity (FCS) and rod and cone functioning are affected in high-risk subjects.
- To identify and grade the risk factors that can lead to diabetes in clinically normal subjects (i.e., those not diagnosed with diabetes).

If colour vision loss is present in subjects diagnosed with diabetes (see Section 2.3.4), it is hypothesised that RG and YB thresholds will be altered in subjects with pre-diabetes (those at a high risk of developing diabetes). It is also anticipated that subjects with pre-diabetes and diabetes will show more significant changes in VA, FCS and rod and cone functioning than the normal population.

Finally, it is hypothesised that identifying changes in visual performance (specifically colour vision performance) will allow for the isolation of risk factors that may be used to detect an increased risk of diabetes.

In summary, this introduction has demonstrated the causes and impact of diabetic retinopathy, as well as the potential relationship between retinopathy and prediabetes. However, it has also shown that the potential use of colour vision loss as a marker for retinopathy in subjects at risk of diabetes needs to be explored in greater detail. Chapter 2 presents a review of the literature relevant to this study, focusing specifically on the role of colour vision assessment in the early stage of diabetic retinopathy. It also provides an account of the strategy used to search the literature and offer a

comprehensive overview of the topic. It identifies gaps in the research and provides a justification for the research undertaken in this study.

2. Literature review

2.1 Introduction and background

This chapter provides a literature review of colour vision screening as a potential tool for the early detection of diabetes and diabetic eye disease (retinopathy). The chapter begins with a general background to the topic, as well as an overview of the search strategy that was used. A justification is provided for the inclusion of specific studies within the analysis. Key results are discussed, with a critical discussion of the available literature and wider practice implications. The chapter identifies gaps in the data and the literature, serving as a justification for this PhD study.

Diabetes is a chronic, progressive condition characterised by elevated blood glucose and poor insulin sensitivity (Qiao et al., 2015). It is a significant global disease that is associated with substantial morbidity and mortality, negatively impacting a person's well-being (ADA, 2003). The condition is linked to a range of negative health outcomes, but the most important of them relate to microvascular and macrovascular complications (Qiao et al., 2015). These complications arise due to a complex process of chronic inflammation and the glycation of proteins in the vasculature and end organs (Gregg et al., 2016). Once pathological disease processes are initiated, they can be challenging to reverse or control, leading to a high level of disease burden that often gets worse over time (Qiao et al., 2015). Therefore, the prevention of diabetes and diabetic complications is an important public health initiative (Qiao et al., 2015). The early identification of at-risk subjects and the prevention of diabetes are the key goals of screening programmes and preventative strategies (ADA, 2003).

Markers of pre-diabetes or high-risk subjects can be used to guide preventative approaches like lifestyle adjustment and targeting risk factors. However, many subjects go on to develop diabetes and associated complications, despite identifying these risk factors (Grundy, 2012). This suggests that more must be done to respond to these risk factors within an effective preventative strategy. It also demonstrates the need to identify additional risk factors that are easy and cheap to screen for (Grundy, 2012).

Early changes in metabolic values (e.g., blood glucose) can provide an opportunity for early intervention in subjects with pre-diabetes, although other markers of diabetes pathology may also be present at the early stages of the disease. These can also be used as markers in screening processes.

Diabetic retinopathy is characterised by a progressive series of pathological changes in the retinal tissue, proportional in gravity to the severity of the diabetes (which is determined by the level of glycaemic control) and the duration of the condition. However, the findings related to diabetic retinopathy are not always consistent in the literature (Ting et al., 2016). Despite these inconsistencies, retinopathy can still be an early marker of diabetes as it is commonly seen in the early stages of the disease (Alder et al., 1997). Retinopathy is a key prognostic marker for eye disease and associated outcomes in subjects with diabetes, but the development of clinical changes associated with retinopathy causes irreversible damage to the visual apparatus, reducing the effectiveness of retinopathy screening as an early preventative strategy.

There are several visual deficits that can pre-date retinopathy and may play a role in predicting it. These include changes in VA, contrast sensitivity, and colour vision (Ismail & Whitaker, 1998). Some changes are noted in subjects with diabetes, such as VA deficits and visual field deficits (Abdel-Hay et al., 2015). Unique observations over the past few decades have suggested that colour vision may be particularly affected in early retinopathy and may precede the development of the classical features of retinopathy (Daley et al., 1987; Shoji et al., 2011; Abdel-Hay et al., 2015). For instance, Shoji et al. (2011) found that colour vision was impaired in a sample of 872 men who had been diagnosed with type 2 diabetes without retinopathy. Meanwhile, Abdel-Hay et al. (2015) suggested that colour vision loss may be a unique manifestation of the early stages of diabetes. They specified the degree and patterns of colour vision changes in type 1 and type 2 diabetes. Therefore, colour vision screening may be a possible strategy for identifying eye disease at an early stage, prior to the irreversible damage that affects subjects with diabetic retinopathy.

The use of colour vision screening to identify early changes associated with the development of diabetic retinopathy in subjects without a diabetes diagnosis (i.e., those at risk of diabetes) represents a novel and potentially important clinical strategy. However, there has been relatively little research into the possibility of screening for colour vision changes in this manner, particularly in at-risk subjects, limiting the clinical applicability of this approach. Indeed, evidence from a systematic review suggests that there are no ideal markers of early eye changes in subjects with diabetes or pre-diabetes, though colour vision data were largely absent from this analysis (Jenkins et al., 2016). It is, therefore, important to evaluate the data on the

potential role of colour vision in the screening or early detection of retinopathy in subjects at risk of diabetes.

2.2 Aim and objectives

This literature review aims to evaluate the current evidence for the use of colour vision screening to identify future retinopathy in subjects at risk of developing diabetes. It has the following objectives:

- To identify and appraise the literature on colour vision changes as a precursor to diabetic retinopathy in subjects already diagnosed with diabetes.
- To evaluate the predictive value of colour vision changes in subjects at risk of developing diabetes.

2.3 Search strategy

To identify the maximum number of relevant studies, a broad literature search was conducted using the following online databases: PubMed, CINAHL, EMBASE, Scopus, ScienceDirect and the Cochrane Database of Systematic Reviews. The same search process was consistently applied across all databases, focusing on the following keywords and Boolean operators: [“retinopathy”] AND [“colour vision”] OR [“colour vision”] AND [“pre-diabetes”] AND [“impaired fasting glucose” OR “impaired glucose tolerance”].

Inclusion and exclusion criteria were applied to make the search more efficient and ensure that the identified articles were relevant. All studies had to be published in

English, for pragmatic reasons (namely, a lack of the necessary time or resources to translate from other languages). No specific criteria were set regarding the publication dates of the articles, although comprehensive studies were favoured in which were afforded more weight in the review and in developing the current study. The analysis also focused on a broad range of subject groups, including subjects with pre-diabetes (IFG or IGT) and established diabetes (type 1 or type 2 diabetes mellitus). Although the focus of the data set was aimed at those with pre-diabetes, there was a significant lack of data on this subject group. This justified the inclusion of subjects with diagnosed diabetes.

The methodological design of the studies was also used to refine the data set. Only quantitative studies were included, due to the nature of the review question. The main studies considered for inclusion were meta-analyses, systematic reviews, randomised controlled trials (RCTs), quasi-experimental studies and cohort studies. Only studies with data on humans were permitted to maintain the clinical relevance of the discussion.

Once the initial data set was identified, several refinements were introduced. This involved reading the title of the article and analyzing the abstract to determine its relevance. This was followed by a full textual analysis of each article to ensure its relevance and check that it adhered to the inclusion criteria. Finally, each study was critically appraised, using a standardised tool for the methodological approach employed in the study, as per the guidance of Aveyard (2014). The Critical Appraisal Skills Programme (CASP) toolkit was used for this purpose, which comprises several checklists that may be applied to specific study methodologies and that have been

widely used in different research settings (Aveyard, 2014). The results of the checklist assessment helped to ensure that the critical appraisal process was reliable and allowed for any significant methodological issues to be identified. These could then be critically discussed, and the study could be considered for exclusion from the final data set.

2.4. Overview of results

There were 34 total possible studies that were identified, followed by the total number of 14 studies relevant to the aim and objectives of the review that eventually remained after the primary types of study excluded from the final analysis; those that focused on animals or diabetic retinopathy in animals, or those that only considered colour vision changes only in advanced stages of the diabetic disease (associated with retinopathy). The refinement process is summarised in Figure 5, which reflects the sequential stages of the preferred reporting items for systematic reviews and meta-analyses (PRISMA, Moher et al., 2009).

The publication dates of these studies ranged from 1992 to 2020. In general, they were cross-sectional analyses of a small number of subjects with or without diabetes who may or may not have shown evidence of retinopathy. The primary types of study excluded from the final analysis were those that focused on animals or diabetic retinopathy in animals, or those that considered colour vision changes only in advanced stages of the diabetic disease (associated with retinopathy).

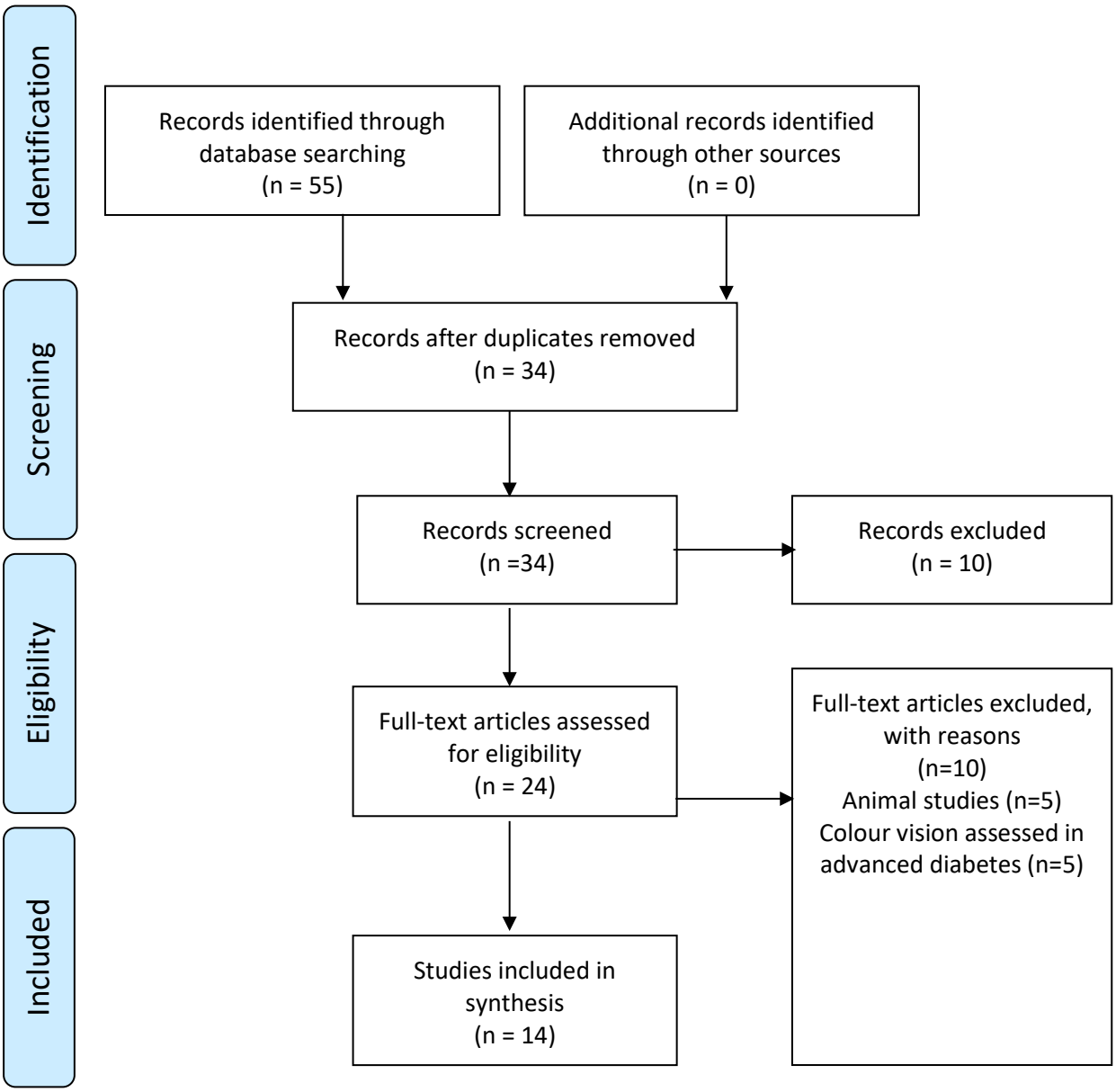

Figure 5: PRISMA flow diagram

The studies were heterogeneous in their methodologies, sample selection and methods of data collection. In general, the studies focused on subjects with a pre-existing diagnosis of diabetes, either with or without retinopathy. Only a minority of studies focused on subjects with pre-diabetes or a high risk of developing diabetes. In general, reviewing these studies suggests that colour vision impairments are under-explored in subjects at risk of diabetes. Indeed, few studies explored the epidemiology or pattern of colour loss in subjects at risk of diabetes (Gella et al.,

2015; Sokolowska-Oracz & Piatkiewicz, 2017). The remaining studies focused on subjects with established diabetes, with or without evidence of retinopathy. Despite the limited evidence regarding subjects with pre-diabetes, some studies offered a comparison between pre-diabetic and control populations (Sokolowska-Oracz and Piatkiewicz, 2017). Most studies focused on subjects with diabetes but without retinopathy. Notably, the literature confirmed that these subjects experience a reduction in colour vision, but there was also heterogeneity in the type of deficiency observed. In the following sections, the findings will be considered in greater detail, based on a critical analysis of the data set.

2.4.1 Subject populations

The first matter to consider is the heterogeneity and characterisation of the subject populations included in the studies. Only a small minority of studies included subjects without evidence of diabetes, reflecting a lack of research focusing on high-risk groups and subjects with pre-diabetes. Vadivel and Vijayamalathi (2016) compared subjects with or without a diagnosis of type 2 diabetes (pre-diabetes versus diabetes) to determine differences in their colour vision characteristics. The study by Sokolowska-Oracz and Piatkiewicz (2017) only included subjects with pre-diabetes. No other studies reported data from these subject groups, with the remaining studies concentrating on subjects diagnosed with diabetes.

Seven studies either included subjects with a diagnosis of diabetes but without evidence of retinopathy or compared two groups of subjects with diabetes, one with retinopathy and one without retinopathy (Feitosa-Santana et al., 2010; Gualitieri et al., 2013; Andrade et al., 2014; Gella et al., 2015; Neriyanuri et al., 2017; Tan et al.,

2017; Vadivel & Vijayamalathi, 2016). Barbur et al. (2012) specifically enrolled subjects without evidence of proliferative diabetic retinopathy. Hardy et al. (1992) included subjects with type 1 diabetes without evidence of retinopathy. In this regard, it is worth noting that while there are important differences in eye disease development in subjects with type 1 and type 2 diabetes, these are not clearly understood. That said, type 1 diabetes is likely to be characterised by a much longer disease process.

Studies also included subjects with a diagnosis of diabetes but for whom the presence or absence of retinopathy was not stated (Tregear et al., 1997; Abdel-Hay et al., 2015). In contrast to other studies, O'Neill-Biba et al. (2010) compared colour vision patterns in subjects with diabetes against those with acute macular degeneration. As macular degeneration may be a later-stage event in subjects with diabetic eye disease, this study afforded the opportunity to elucidate differences in populations based on the timing and the different stages of eye disease. Also, Wolff et al. (2015), unlike other researchers, compared colour vision loss in diabetics with retinopathy with colour vision loss in those without retinopathy. However, critical consideration is needed to assess eye disease characteristics in subjects with established diabetes, as these findings may have limited relevance in the pre-diabetes population.

2.4.2 Study designs

There was marked heterogeneity in the design of the studies, reflecting several limitations and challenges associated with the assessment of colour vision in subjects with pre-diabetes or established diabetes. Prospective study designs (studies focused on subjects with the potential to develop diabetes) were noted in the literature,

although this did not make up a large proportion of the data set. Tregear et al. (1997) performed a prospective study, although there was a lack of clarity regarding the timeframe of the data collection points. Similarly, Sokolowska-Oracz and Piatkiewicz (2017) evaluated subjects with follow-ups over nine months to determine changes in colour vision associated with diabetes.

The majority of the studies reviewed included cross-sectional data (Hardy et al., 1992; O'Neill-Biba et al., 2010; Feitosa-Santana et al., 2010; Barbur et al., 2012; Gualitieri et al., 2013; Andrade et al., 2014; Abdel-Hay et al., 2015; Gella et al., 2015; Wolff et al., 2015; Vadivel & Vijayamalathi, 2016; Neriyanuri et al., 2017; Tan et al., 2017). These studies provided varying levels of insight into the link between colour vision and the characteristics of diabetes. However, they were limited in their ability to establish causation between variables or identify risk factors. This was primarily because none of them took a longitudinal approach.

2.4.3 Colour vision assessment in diabetic subjects

A wide variety of procedures were employed to assess colour vision, suggesting uncertainty on what the ideal method is for assessing colour vision in the subject groups. Colour arrangement tests were commonly employed, particularly in the older studies. For instance, the Farnsworth–Munsell 100 (FM 100) hue test was used by Hardy et al. (1992), Andrade et al. (2014) and Vadivel and Vijayamalathi (2016). The FM D-15 colour test and colour confusion score (CCS) calculations were used by Wolff et al. (2015), Sokolowska-Oracz and Piatkiewicz (2017) and Feitosa-Santana et al. (2015). The latter also employed the Cambridge Colour Test (CCT).

Computerised testing, with a more nuanced analysis of colour axes, was also used in some studies. Tregear et al. (1997) used computer-controlled, cathode-ray-tube-based tests of chromatic contrast and threshold estimates across all colour axes. The CAD test (see Section 3.6.1 for more details) was used in several studies to assess subjects diagnosed with diabetes. Abdel-Hay et al. (2015) used this test for monocular assessment. O'Neill-Biba et al. (2010) employed it binocularly. Barbur et al. (2012) used it binocularly alongside macular optical coherence tomography (OCT) and fundus photography. The use of multiple testing techniques may be beneficial in measuring colour vision loss and comparing the loss to structural changes observed in imaging techniques.

There were important variations in the methods used to analyse colour vision and the characteristics of the subject populations. Indeed, non-digital FM 100 and D-15d tests have been largely superseded by computerised versions of these tests or by other digital tests, which provide greater insight into the pattern of colour vision loss (O'Neill-Biba et al., 2010). The data in the present review are based on the use of different tests, leading to different outputs in the descriptions of patterns of colour vision loss and affected axes.

As mentioned, one of the most widely used tests within occupational environments is the CAD test (see Section 3.6.1). This test can be easily combined with other tests for VA and rod and cone sensitivity. This further supports the use of this approach in practice using the Advanced Vision and Optometric Tests (AVOT), developed by City, University of London (2017; 2018). It should be noted that the high discriminatory capacity of the CAD for axis-specific colour vision loss may overcome potential

weaknesses observed in the older tests, including colour arrangement tests such as the FM 100 and the D-15 (Costa et al., 2007; Gualtieri et al., 2013). Therefore, the method chosen method for assessing colour is an important consideration when analysing the findings reported in the studies collected here. The ideal colour vision test needs to be able to assess both RG and YB chromatic sensitivity, while also having high sensitivity and high specificity.

2.4.4 Patterns of colour vision loss in diabetes

There is clear evidence that colour vision is significantly worse in diabetic subjects with diabetic retinopathy compared to control groups (healthy members of the public without evidence of diabetes). In general, despite using a variety of different tests, the studies detected deficiencies in colour vision in subjects with diabetes compared to those without diabetes. For instance, Hardy et al. (1992) found that FM 100 scores were significantly higher in subjects with diabetes compared to control subjects (86.6 versus 28.2; $p < 0.001$). Wolff et al. (2015) noted that in comparison with control subjects, colour vision loss was consistently affected in diabetic subjects regardless of the presence of retinopathy. Specifically, chromatic sensitivity was reported in 41% of subjects with diabetes without retinopathy and 48% of subjects with diabetes and retinopathy, whereas it was reported in just 3% of control subjects. However, the small sample size ($n = 84$) in Wolff's study limits the strength of these findings. The molecular genetics study by Gella et al. (2015) revealed that 39.5% of subjects without retinopathy had colour vision loss. The study by Sokolowska-Oracz and Piatkiewicz (2017) showed that colour vision loss measured with the D-15 was higher in subjects with pre-diabetes (those who had positive IFG and/or IGT) compared with control subjects (8.33% and 0%, respectively). However, these results did not achieve

statistical significance. Furthermore, this prevalence rate of colour vision loss in this study is much higher than that reported in other studies. The reasons for this are unclear, although it perhaps suggests that the sample selection process may have been biased; further data would be needed to account for this high level of colour vision loss.

Several studies also explored the patterns of colour vision loss, not just the evidence of colour loss. Colour vision loss patterns relate to specific defects that affect either the RG channel or the YB channel. Occasionally, the defects can affect both channels, which is known as ‘diffuse loss’. Tan et al. (2017) found that 22% of subjects with diabetes and without retinopathy had colour vision defects that were principally tritan (or YB channel) in nature. These findings supported those of Tregear et al. (1997), who compared 305 eyes of diabetic subjects with those of control subjects and noted derangement in colour vision axes in subjects with diabetes, predominantly along the YB axis. Vadivel and Vijayamalathi (2016), who used the FM 100 test to compare 85 subjects with type 2 diabetes to control subjects, found that YB defects were more common in diabetic subjects without retinopathy. Although the subjects in all of these different studies were not at the same stage of diabetes and did not share the same risk factors or complications for diabetic eye disease, it is notable that YB disturbances were primarily noted across all of them.

However, other studies found that colour vision loss was distributed equally along both the YB and RG axis, suggesting that colour vision loss may not be specific to the YB axis (O’Neill-Biba et al., 2010). It is important to note that the involvement of the optic nerve may also modify the pattern of colour vision loss, reflecting changes

in the severity of diabetes over time and/or specific characteristics of the disease process in different individuals. This may account for variations in colour vision loss patterns seen in the included studies, particularly given the heterogeneity of the subject groups assessed. For instance, Abdel-Hay et al. (2015) found significant RG loss in 72% of subjects and YB loss in 65% of subjects with type 1 or 2 diabetes. Using the FM D-15 test, Gualtieri et al. (2013) found colour discrimination losses along all three axes (tritan, protan and deutan), with a slightly higher degree of protan loss in the test group compared to the control group. Feitosa-Santana et al. (2010) compared diabetic subjects with the best quality eyes with those with the worst quality eyes. They found diffuse loss in colour discrimination in 13.3% of the subjects with the best eyes and 29% of the subjects with the worst eyes. Similarly, in a study carried out in Moorfields Eye Hospital in Dubai, Barbur et al. (2012) noted diffuse colour loss in diabetics without proliferative retinopathy (approximately 70% of subjects showed signs of chromatic sensitivity loss). Studies by Andrade et al. (2014) and Neriyanuri et al. (2017), both of which used the D-15 test, also identified diffuse colour loss that was not specific to one axis.

As mentioned previously, the mechanisms underlying specific patterns of colour vision loss are complex and only partially understood. Lammer et al. (2016) found that cone photoreceptor irregularity progresses during diabetes, perhaps accounting for the diffuse loss of colour vision commonly observed in diabetics. Changes to the architecture, distribution or density of photoreceptors may be secondary to neuropathic processes, particularly as changes in colour vision may be independent of vasculopathic changes in the eye (Tam et al., 2012). The diffuse pattern of colour vision loss suggests that it may be caused by a specific mechanism of retinal

neuropathy, rather than filtering (as in lens discolouration) or targeting of specific cone subtype function (Lammer et al., 2016).

Curtis et al. (2009) noted that colour vision loss may pre-date the onset of clinical diabetes and may occur before there is any evidence of retinopathy. This highlights the early nature of this deficit in the disease process. Although colour vision is subclinical in nature and seldom reported by subjects in the early stages of diabetes or pre-diabetes, there is evidence for colour vision deficits in these populations (Malukiewicz et al., 2009). The mechanism accounting for early changes may be the same as the one present in more advanced stages of eye disease, highlighting the sensitivity of colour vision mechanisms in diabetic or pre-diabetic subjects to metabolic or neuropathic changes (Curtis et al., 2009). Indeed, retinal nerve fibre layer thinning has been observed in subjects with pre-diabetes (through the use of OCT), and this is associated with the potential for colour vision loss (De Clerck et al., 2017).

Colour vision defects may, however, be indicative of other pathological mechanisms. For instance, Tan et al. (2017) found that advanced age, low levels of education, a longer duration of diabetes, poor glycaemic control, exposure to tolbutamide, and lower mean cholesterol were all associated with colour vision loss. Gella et al. (2015) found that women were more likely to experience losses in colour vision, as were people with increased ocular pressure or increased heart rates. Gella et al. (2015) also found that people with elevated high-density lipoprotein cholesterol levels demonstrated less evidence of colour vision loss. A relationship was found between colour vision loss and the presence of cataracts, corneal surface disorders, arterial

narrowing and angiopathy in subjects with pre-diabetes, compared with control subjects (Sokolowska-Oracz & Piatkiewicz, 2017). Cataracts may be associated with a pronounced loss in the YB axis due to pigmentary changes in the lens of the eye (Fristom & Lundh, 2000). Furthermore, one of the most significant factors associated with impaired colour vision was prescription of tolbutamide, which increased the risk of impairment by 3.79 times (Tan et al., 2017). No other diabetic medications were associated with an increase in colour vision impairment. The reason why tolbutamide should have this effect is not clear, but it has been associated with YB colour vision loss in multiple studies (Thompson et al., 1979; Pavan-Langston, 2009). Tolbutamide is an older class of sulphonylurea; the replacement of tolbutamide with newer agents may reduce the risk of colour vision deficits (Tan et al., 2017). Therefore, there is no indication that the effects of tolbutamide on glycaemic control increase the risk of colour vision defects, rather it seems that this drug may result in suboptimal diabetic management and poor glucose control more generally. Taken together, these studies suggest that clarifying the pattern of colour vision loss would provide further insight into the mechanisms that lie behind colour vision defects and reductions.

Another possible explanation for the differences in observation regarding the patterns of colour vision loss relates to the assessment tool selected in each of the different studies (Gualtieri et al., 2013). For instance, the FM 100 is less able to identify RG vision loss than other tests, such as the CAD test (Gualtieri et al., 2013). This implies that previous studies that mainly identified tritan-type losses may not have provided an accurate description of the colour vision defect that was present. Mechanisms such as the susceptibility of specific cone subtypes and lens yellowing may also explain axis-specific colour vision loss (Tregear et al., 1997). Verriest's classification of colour vision defects suggests that RG-axis effects are associated with damage to the

inner retina, whereas YB-axis defects are attributable to damage to the photoreceptors (Pokorny & Smith, 1986). Furthermore, hypoxic conditions and cellular apoptosis in the retina and ganglion cells have also been shown to lead to colour vision loss (Barbur and Connolly, 2011). This is potentially triggered by vasculopathic or neuropathic processes linked to diabetes and/or comorbid conditions. Therefore, multiple mechanisms may operate in concert to yield different patterns of colour vision loss, reflecting the heterogeneity of diabetic retinopathy and the characteristics of different subjects. It should be noted, in this regard, that subjects with other conditions not associated with the main focus of this study have been excluded, making it easier to examine and isolate the effects of diabetes on colour vision.

Several studies have also shown that uncontrolled glycaemic levels can cause colour vision loss (Tan et al. 2017; O'Neill-Biba. 2010; Tregear et al., 1997). O'Neill-Biba et al. (2010) found that the severity of colour vision loss in diabetic subjects was correlated with hyperglycaemic control. Tregear et al. (1997) found similar results for YB loss, but this correlation was not significant when lens yellowing was taken into account. These studies suggest that poor glycaemic control, which is strongly linked to the development of diabetic retinopathy and other complications, may also be linked to a deterioration in colour vision. However, there have also been some studies that did not find any significant correlation between risk factors for diabetes and changes in colour vision. Kessel et al. (1999) proposed that the colour vision deficits seen in subjects with diabetes are largely attributable to lens yellowing. They found minimal differences in the colour vision scores for diabetic and non-diabetic subjects who had received cataract surgery. Furthermore, while lens yellowing may contribute to changes in colour vision (Lopez et al., 2002), this may not account for the early,

subclinical changes in colour vision noted in subjects with pre-diabetes or those with early-stage diabetes. Using the FM-100 test, Hardy et al. (1992) found that colour vision in subjects with type 1 diabetes without retinopathy was not correlated with the length of the diabetes diagnosis, blood glucose levels, or glycated haemoglobin levels. Similar results were reported by Andrade et al. (2014), who also used the FM-100 test. Abdel-Hay et al. (2015) found no correlation between chromatic sensitivity (measured using the CAD test) and the duration of diabetes, type of diabetes (type 1 versus type 2), or the age of the patient. These findings supported those of Barbur et al.'s (2012) study, which reported no significant impact as a result of age, gender, duration of diabetes, diabetic control or central macular thickness.

Recently, Neriyanuri et al. (2017) used the D-15 test on 743 diabetic subjects and found a positive correlation between colour vision and diabetic neuropathy in the absence of retinopathy ($p = 0.04$). No other studies have identified neuropathy as a specific risk factor for colour vision changes, although the electroretinographic results of Gualtieri et al.'s (2013) study suggested that colour vision deficits may be related to abnormalities of the inner retina. The significance of this finding was unclear, however, and larger samples would be needed to confirm the specific axes that were affected at different stages of colour vision loss in the diabetic disease process.

In the following section, the use of colour vision as a screening tool for diabetes in subjects with pre-diabetes will be considered, focusing particularly on how the risk factors of diabetes influence the progression of retinopathy and diabetes pathogenesis.

2.4.5 Colour vision screening in diabetes and pre-diabetes

Given the evidence that colour vision changes are an early sign of diabetes and pre-diabetes, colour vision screening could be an effective way of identifying people who are at a high risk of developing the disease. However, relatively few studies have explored the prognostic or progressive nature of colour vision as a tool for predicting future retinopathy or diabetes (Wolff et al., 2015; Sokolowska-Oracz and Piatkiewicz, 2017). Sokolowska-Oracz and Piatkiewicz (2017) noted that changes in colour vision and other ocular changes were consistent in subjects with pre-diabetes and control subjects over nine months; retinopathy increased in both groups, with no clear differences between them. Wolff et al. (2015) found that colour vision loss was correlated with neuropathic changes to retinal tissue in subjects with diabetes who had no evidence of retinopathy. This may suggest that colour vision changes are a sign of early retinal damage. Nevertheless, both of these studies were limited by their small sample sizes and their lack of a robust, longitudinal approach to colour vision assessment. The latter would be necessary to establish is required to establish a clear timeline for colour vision changes within the context of the development of diabetes. Furthermore, the role of specific risk factors in influencing colour vision loss may be overlooked if those risk factors are not robust and not relevant to the population being analysed (Tan et al., 2017).

Moreover, doubts have been raised regarding the value of colour vision screening in subjects with diabetes. Tan et al. (2017) found that colour vision was only significantly affected in subjects whose diabetes had lasted at least six years. They also questioned the cost-effectiveness of colour vision screening in this population. However, the cross-sectional nature of this study means that it does not contradict the

idea that early changes in colour vision may have been evident in subjects before they were diagnosed with clinical diabetes. That said, cost-effectiveness is an important consideration when it comes to colour vision screening; the cost of specialist colour vision assessments should be compared to that of carrying out blood tests or testing for other indicators. Colour vision screening should only be recommended if it is likely to have a positive impact on subjects after the screening, for instance by allowing for preventative or early-stage interventions (Rein et al., 2012). It is hoped that the data provided by the current study will demonstrate the importance of testing for colour vision changes in subjects with pre-diabetes or early-stage diabetes, while also establishing mechanisms that will help to make the process cost-effective.

There is evidence to suggest that changes in ocular function are common in subjects with pre-diabetes. Recently, Sahin et al. (2018) found specific macular and peripapillary changes in pre-diabetes using OCT imaging. They also found that macular thinning as part of a neurodegenerative process was more common in subjects with pre-diabetes than in control subjects. Their findings have been confirmed in the Maastricht Study of subjects with pre-diabetes or type 2 diabetes without retinopathy, suggesting that macular thinning is an important event that precedes diabetic retinopathy (De Clerck et al., 2018).

2.4.6 Visual performance tests

The literature review highlighted several ways of testing visual performance and colour sensitivity. The methods used for testing are described in Section 3.6 of the following chapter. The results are also described in Section 4.4. This section provides

a brief summary of the tests that were used. The results from these tests were cross-correlated with the information about subjects' risk of developing diabetes.

The first test that was carried out was a colour assessment diagnosis (CAD), which was used to assess RG and YB chromatic sensitivity. Rod and cone sensitivity were examined using the Flicker-Plus test, which examines the spectral, spatial, and temporal properties of vision mediated by rods and cones. Finally, an Acuity-Plus test was performed to measure VA and FCS. This test measures people's sensitivity to contrast, which helps to assess visual performance at different spatial frequencies and luminances.

2.4.7 Critical evaluation and implications

The findings of the studies included in this review should be carefully considered with respect to their study designs used and the populations analysed. Cross-sectional studies using different subject populations, including subjects with pre-diabetes or diabetes, as well as some with or without diabetic retinopathy, create a heterogeneous population that is difficult to analyze. Moreover, many of the studies have small sample sizes, reducing the reliability of the findings, and many also lack details on the statistical test protocols and the assumptions underlying the tests.

The gaps in the literature suggest that changes in colour vision associated with diabetes must be carefully analyzed, with a focus on the broad risk factors that influence visual functioning over time, as well as the factors that are associated with diabetes and changes in colour vision. A lack of consideration of many vascular risk factors may lead to incorrect conclusions being drawn about certain correlated

effects. When it comes to any cross-sectional study, it is important not to assume that correlation equals causation. That said, due to the difficultly of obtaining longitudinal data about the diabetic population and those at risk of diabetes (but without a clinical diagnosis), cross-sectional data may still be valuable for exploring the link between colour vision and diabetes in the near future.

The findings of the review suggest that risk factors may be indicative of colour vision loss in subjects with early diabetes and possibly pre-diabetes as well. However, further clarification about the potential utility of colour vision screening as an early way of identifying diabetes or diabetes-associated eye disease is necessary. Therefore, this study will cross-verify the risk factors that correlate to both colour vision loss and diabetes.

2.5 Limitations

There are some limitations to the literature review process that was conducted, as well as to the studies that were included. Firstly, the review was carried out by a single reviewer, which may have introduced bias into the data collection and analysis. However, the use of a structured and transparent framework was intended to reduce this risk. Secondly, relatively few studies on the review topic were located, and there were very few focused on subjects at risk of diabetes. This made it difficult to provide a detailed analysis of the data set, but it did make it possible to identify gaps in the knowledge base. Finally, the quality of the studies varied substantially, leading to heterogeneity in the data set, which made it difficult to synthesise all of the data.

2.6 Summary and knowledge gaps

This literature review has highlighted the link between loss of colour vision and the early features of diabetes and diabetic eye disease. It has identified studies that have measured changes in chromatic sensitivity using a variety of tools and assessment procedures. These colour vision changes may be tritan in nature or may reflect a more diffuse pattern of colour vision loss. The findings reported in the literature vary depending on the population studied and the methods used to assess colour vision. In general, the literature shows that chromatic sensitivity changes in diabetics are likely the result of a series of heterogeneous and complex events.

The studies proposed several mechanisms to account for the loss of chromatic sensitivity in diabetics. These include pre-retinal changes (e.g., lens yellowing), age-associated changes in cone function or morphology, the effects of hyperglycaemia, and combinations of vasculopathy and neuropathy in the retina. Emerging data tend to support a role for early photoreceptor dysfunction in diabetes, which may be a precursor event for diabetic retinopathy.

The findings of the included studies suggest that colour vision loss is a characteristic part of the diabetes disease process. The findings suggest that the pattern of colour vision loss often involves the YB axis, however, some studies have shown that the loss is diffuse in nature and/or involves multiple axes. Where colour vision loss was noted in subjects with diabetes, similar findings appeared in subjects at risk of developing diabetes. This suggests that colour vision loss may be an early indicator of vision deterioration, preceding the onset of clinical diabetes. However, the studies

were less consistent regarding the pattern of colour vision loss and the future deterioration of vision in subjects with diabetes.

Despite the evidence highlighting the link between diabetic disease processes and the loss of colour vision, there remain several unanswered questions in this field. Firstly, the value as a screening process or a preventative measure of assessing chromatic sensitivity changes in subjects with diabetes is unclear. Although these changes may occur early in the disease process, inconsistencies in the studies that have been conducted thus far make it impossible to identify a definitive link between colour vision changes and the onset of diabetic eye disease. As a result, it is not clear whether assessing colour vision would make it possible to classify subjects based on their risk of diabetic retinopathy, or whether it could be used to identify subjects in need of targeted strategies to prevent visual loss.

Secondly, the association between colour vision loss and other types of visual abnormalities in subjects with diabetes remains unclear. Often, VA is preserved in subjects with colour vision loss, even when subjects present abnormal results to other visual tests. Understanding how vision is affected in general, while focusing on chromatic sensitivity, will ensure that all mechanistic processes are understood and evaluated in the context of holistic patient care. Therefore, tests of VA (such as the Acuity-Plus test), under both photopic and mesopic conditions, may help to establish baseline levels of visual function. This will be explained in more detail in the following chapter.

Thirdly, the studies show that the precise pattern of colour vision loss in diabetes can vary. It can result in focusing on tritan, deutan or protan loss, or it can produce a diffuse pattern of colour vision loss. The type of vision loss that occurs in diabetic subjects should be carefully evaluated and compared with age-matched subjects without diabetes to establish the type of colour vision loss. These evaluations should also consider the potential link with other pathological mechanisms and the clinical characteristics of the subjects involved.

Finally, the factors that may modulate or attenuate colour vision loss in subjects with diabetes remain unclear. This is particularly important when it comes to diabetes medication, as optimal glycaemic control may be an achievable means of preventing colour vision loss and, potentially, the further retinal damage associated with the disease. Appreciating the value of glycaemic medications in moderating colour vision loss, either positively or negatively, should form an important aspect of future studies. This will form part of the evidence base used to tailor treatment strategies to subjects with diabetes or pre-diabetes who with early visual changes, including chromatic sensitivity changes.

2.7 Conclusion

In summary, this literature review has explored the use of colour vision testing as a means of evaluating early diabetic retinopathy in diabetic and pre-diabetic subjects. It has revealed that there is a paucity of relevant data to suggest whether colour vision is disturbed in persons with pre-diabetes or who are at a high risk of diabetes. However, some data suggest that subclinical deficits in colour vision can be identified in these subjects. These findings are supported by studies that demonstrate specific colour

vision loss in subjects with diabetes but without evidence of retinopathy. The reviewed papers suggest that certain risk factors may be linked to colour vision loss in early diabetes and pre-diabetes, although it is not clear which of these are the most clinically significant. Most importantly, the review demonstrates that there is a need for further data on colour vision loss that focuses on patterns of loss and axis-specific loss in pre-diabetic subjects. This would help to demonstrate whether colour vision screening could be used as a way of predicting the development of diabetes in this population.

As mentioned in chapter 1, the prevalence of diabetes is high in the Gulf countries and that the population of Kuwait has become highly susceptible to diabetic retinopathy. Colour vision assessment is a sensitive form of measurement that has the potential to be an important way of identifying risks in pre-diabetic screening. The current study hopes to develop a protocol based on loss of chromatic sensitivity to help tackle the alarming situation regarding diabetes in Kuwait.

3. Methods and Protocols

3.1 Overview

The aims and objectives of this study were developed in response to the paucity of evidence highlighted in the literature review. This chapter provides an insight into, and a justification for, the methods employed during the research project. It outlines the design of the research project, the nature of the data collected, and the techniques used for data analysis. It also discusses the protocols used for all the visual testing procedures that were employed, offering justifications for them with reference to the scientific literature.

Those aims were refined based on an overall evaluation of the 213 subjects chosen for the study. Of those subjects, 150 were at a high risk of developing diabetes (the target group), 40 were normal subjects who were neither diabetics nor had a high risk of developing diabetes, and 23 were subjects who had already been diagnosed with diabetes. As the aims specifically relate to the testing protocols undertaken, it is important to consider their implications in this context. The first aim was to examine the type of colour vision loss in subjects already diagnosed with diabetes. The purpose of this was to confirm the findings of previous studies and then to compare these with those for the high-risk subjects in this study. The second aim was to investigate RG and YB thresholds in subjects at a high risk of developing diabetes. At the same time, a wider assessment of these subjects' visual functioning was also conducted, to assess whether the development of diabetes was the most likely cause of any changes in visual functioning. These aims involved careful consideration of data normality in each population to select appropriate statistical tests for within- and

between-group comparisons. The final aim was to identify and grade the risk factors in clinically normal subjects that can lead to diabetes by correlating these risk factors with colour vision loss. The remainder of this chapter outlines the design of the study, the characteristics of the sample population, the testing protocols, and the methods of data analysis that were used in pursuit of these aims.

3.2 Design and approach

As mentioned, the overarching aim of this thesis was to establish the significance of the loss of colour vision in Kuwaiti adults who were at a high risk of developing diabetes. If a significant proportion of subjects in this cohort were affected, then accurate assessments of RG and YB thresholds would be important risk factors in pre-screening for diabetes. An additional aim of this thesis was to explore and examine the visual performance of subjects who had been diagnosed with diabetes with no retinopathy, thereby confirming the findings of previous studies (see Section 2.4.4).

There are many ways of designing a study to determine the link between clinical symptoms and assessment outcomes, including longitudinal and cross-sectional approaches. Longitudinal data can be a valuable way of monitoring changes in the levels of risk over time, but longitudinal studies often require extensive amounts of data and repeated measurements. They also require significant amounts of time and can be technically challenging (Wood et al., 2011). By contrast, cross-sectional studies are easier to perform as they only collect data at a single time point, but they do not provide detailed information about the causal link between variables and outcomes; they only demonstrate potential correlations (Wood et al., 2011). For the

current study, it was thought that a cross-sectional approach would have the potential to determine a correlation between colour vision and diabetic status, while also providing a simple strategy for assessing the study's participants. A cross-sectional design was also favoured to ensure that the study could be feasibly completed within the required timeframe.

To provide a clear comparison, the study evaluated two cohorts of subjects: (i) subjects with pre-diabetes and (ii) subjects with diabetes. This was the most direct and logistically simple way of evaluating the two cohorts simultaneously. The data collection took place in Kuwait at the Al Shalahi Specialized Centre (hereafter 'the Centre'). Multiple visits were conducted so that as many subjects as possible could be examined.

3.3 Ethical approval

Ethical approval was obtained from City, University of London and from the Ministry of Health in Kuwait (see Appendix D). Appendix B and Appendix C provide copies of the participant information sheet used for this study and the corresponding consent form that was completed by all participants.

3.4 Inclusion and exclusion criteria

The inclusion and exclusion criteria for participation were as follows: subjects in the pre-diabetic group had to have at least three clinically accepted risk factors for diabetes, as detailed by the ADA (see Section 1.7, Table 2).

The following additional definitions were applied to the risk factors as inclusion criteria:

- Elevated HbA1c (glycated haemoglobin) levels between 5.7% and 6.4%. The ideal level for HbA1c is 42 mmol/mol or less, below 5.7%. Diabetics have 48 mmol/mol or more, above 6.5% (*Guide to HbA1c*, 2019).
- High blood pressure over 130/80 mm Hg (systolic/diastolic blood pressure), as stipulated in the 2017 *High Blood Pressure Clinical Practice Guideline* (Whelton et al., 2018).
- Elevated BMI ≥ 25. According to the National Institutes of Health (NIH, 1998), a BMI in the range of 18.5 to 24.9 is ideal. However, a BMI from 25 to 29.9 is considered above the ideal range, and a BMI of 30 or more represents obesity.
- Elevated fasting blood glucose levels in the range of 5.5 to 6.9 mmol/l (100 to 125 mg/dl). Normal fasting blood glucose levels are below 5.5 mmol/l (100mg/dl). Diabetic subjects have levels greater than 7 mmol/l (126mg/dl) (American Diabetes Association, 2009).

Every subject underwent a clinical ophthalmological pre-examination. This involved a VA assessment using a Snellen chart, fundoscopy, slit-lamp examination and tonometry. The purpose of this was to ensure that none of the participants included in the study had any ocular or retinal abnormalities. However, OCT imaging was not carried out on the subjects due to the protocol followed at the Centre, which meant that it was not possible to include that this in this study. Although OCT is useful in diagnosing many ocular diseases (retina and optic nerves), one of its disadvantages is that it is not done routinely. As such, it is not easily accessible and is only available in

tertiary specialist eye hospitals. CAD, on the other hand, is readily available and easy to access. Another issue with OCT is the amount of time it requires. The process involves taking a series of images over time, and the patient has to lie still to avoid affecting the quality of the images. The machine also requires a professional to operate it and accurately place the image on the desired field as this can be easily missed. Therefore, it was thought that it would be more convenient to use CAD test that was more sensitive, more specific, more efficient, less costly, and simpler to use.

The clinical examination was used to exclude subjects who showed signs of ocular diseases or systemic conditions that were associated with ocular defects or visual disturbances. This was to prevent the data on diabetic and prediabetic subjects from becoming muddied by pre-existing visual defects. Subjects were also excluded if they had known retinal diseases, abnormal fundus colour or presence of drusen, all of which can affect colour vision and might have interfered with the primary aim of the study. Participants with significant cataracts, as assessed using the Chylack LOCS III lens opacities classification system, were also excluded (Chylack Jr et al., 1993). This system consists of six slit-lamp images for grading nuclear cataracts and opacity, five retro illumination images to grade cortical cataracts, and five retro illumination images to grade posterior subcapsular cataracts. It is widely used in clinical practice and provides a detailed and reliable insight into lens classification and cataract severity. As cataracts can affect colour vision, this was a valid reason for excluding these subjects from the study (Tregear et al., 1997). Subjects diagnosed with age-related macular degeneration (AMD) were also excluded from this study.

3.5 Geographical location

Subjects were selected from the adult population of Kuwait, a region of interest given the high prevalence of diabetes in the area. All subjects were recruited from an eye clinic (poly clinic) at the Centre in Kuwait. Due to the nature of this clinic, it should be acknowledged that the subjects recruited may not have been representative of the wider Kuwaiti population.

The number of participants that took part in this study was contingent on the author's ability to travel to Kuwait and access the facilities at the Centre. Blood tests (for evaluating inclusion criteria) were carried out at the same Centre, and all the subjects who met the inclusion criteria were approached and invited to participate in the study. All participants had to provide informed written consent in the presence of the researcher before participating in the study (See appendix B). They were informed that they could withdraw from the study at any point for no reason.

3.6 Data-collection procedures

All data-collection procedures were performed in a consistent and reproducible setting, using defined protocols and procedures. This was intended to minimise the potential for bias during the testing process and to optimise the data-collection process. All the examinations were performed in a room in the Centre that was suitably equipped. Privacy was maintained during the testing process, and the equipment was routinely checked and calibrated between tests to avoid errors.

The Centre in Kuwait, described above, is a dedicated screening centre for diabetes. It is visited by large numbers of people every day. To avoid non-compliance of the study participants, all the tests were performed on the same day, allowing for appropriate breaks when needed. The appointments were not necessarily at the same time each day; they were arranged at a convenient time for the participant.

Subjects were recruited from the Al Shalahi specialised centre in Kuwait. Only adults were eligible for recruitment (further inclusion and exclusion criteria are defined in Section 3.4). All subjects were already attending the centre in Kuwait, so recruitment was exclusively focused on the centre. Posters and leaflets were strategically placed in the centre. They invited prospective participants to enrol in the study, provided they met the inclusion criteria, which were outlined in the documents.

The participants were encouraged to approach healthcare staff or contact the researcher directly if they wanted to participate in the study. Participants who responded in this way (by email or phone) were informed about the study in greater detail; either information packs were sent to the patients or they were asked to collect information packs from the centre. Also, once the prospective subjects agreed or declined to participate, they were asked to confirm by email or phone. Then, the participants were asked to attend the centre to discuss the study and sign the written consent form, assuming they were happy to proceed.

All the data collection took place in Kuwait, at the specialist centre described above. This centre screens a large number of subjects for diabetes due to the high prevalence of the disease in this region.

All the data collection took place on the same day for each participant, thereby minimizing confounding factors that might have arisen due to differences in the data collection days or the times of collection.

A questionnaire was developed to assess patients' key demographic and clinical information that was relevant to quantifying their diabetes risk factors (see Appendix A). the questionnaire was based on established risk factors for diabetes and was designed by the researcher based on similar questionnaires in the published literature (e.g. Herman et al., 1995).

Initially, demographic information was collected for all participants using the questionnaire (Appendix A: study questionnaire). This provided rapid and comprehensive data about all participants and served as a baseline data set for the study. The questionnaire covered the following areas: age, body mass index, physical activity levels, dietary factors, hypertension, blood glucose assessment (i.e., did the patient have a diabetes diagnosis), family history of diabetes, and screening for known colour vision deficiency. All the data collected from the participants were made anonymous (by assigning participant numbers) and matched to the participant number used in further testing.

The aim of the questionnaire was to assess the dietary habits and risk factors of diabetes (based on the information from the American Diabetes Association, 2009). This questionnaire was part of a PhD study to establish how and whether the loss of

colour vision and other visual parameters affects a significant number of people at risk of developing diabetes.

The questionnaire and the results of the blood tests were used to determine the presence of the diabetic risk factors, as defined by the ADA (see Section 1.7, Table 2). It is worth noting that not all blood tests (for example, HbA1c tests) were performed on subjects who were not considered clinically diabetic.

After completing the questionnaire and being assessed for at least three of the risk factors for diabetes, the participants were subjected to three tests to measure their visual performance (particularly, their chromatic sensitivity), their VA and FCS, and their rod and cone sensitivity. These tests were part of the Advanced Vision and Optometric Tests (AVOT), developed by City, University of London (2017; 2018). They are described below along with the protocols employed.

3.6.1 Colour Assessment and Diagnosis (CAD) test

The CAD test is arguably the most well-known of the AVOT tests. It is currently the accepted test for assessing colour vision requirements in pilots and is used by the Civil Aviation Authority (CAA) in the UK (see the colour vision guidance material) and the European Union Aviation Safety Agency (EASA). The CAD test has also been used to assess colour loss in subjects with acquired visual deficiency (Barbur et al., 2004; Moro et al., 2007; O'Neill-Biba et al., 2010). The CAD test is based on a spatiotemporal masking technique that isolates the use of colour signals (Barbur et al., 1991; Barbur et al., 1993; Barbur et al., 1994; Barbur et al., 1997; Birch et al., 1992) and a visual psychophysical procedure for measuring thresholds for a colour

response. The CAD test has been recently described in detail by Barbur and Rodriguez-Carmona (2017).

The standardised version of the CAD test displays moving stimuli buried in dynamic luminance contrast noise, and the output is measured in terms of RG and YB colour thresholds (Barbur & Rodriguez-Carmona, 2017) that are approximately linearly proportional to the cone contrasts generated by the coloured stimulus (Rodriguez-Carmona et al., 2012). A colour-defined stimulus (see Fig. 6) is generated against a daylight (D65) background and is presented to the subject as it moves diagonally in one of four possible directions. Following each presentation, the subject must press one of four buttons (arranged in a square) to indicate the direction of the movement. 16 different colour directions are examined (12 for RG and 4 for YB) to measure both the severity and the type of colour vision loss. The pattern of RG and YB colour loss provides information about whether the deficiency is congenital or acquired. For example, elevated RG thresholds and normal YB would be indicative of congenital colour deficiency.

The CAD test also takes into account the effects of normal ageing by adjusting the RG and YB thresholds based on a person's age. For people aged 20 and up, there is a linear increase in thresholds of ~1% for RG and ~1.6% for YB (Barbur & Rodriguez-Carmona, 2017).

A full CAD test, lasting between 12 and 20 minutes, was carried out for each participant to explore the potential patterns of colour vision loss. To save time, the CAD test was performed binocularly. Monocular testing would have taken too long, particularly when combined with the other necessary tests. Furthermore, monocular

testing is generally more appropriate for studies involving correlations between structural changes to the retina and changes in colour vision, but this is rare in prediabetic subjects, who were the focus of this study (see Section 5.6).

The CAD test was performed under conditions of ambient lighting, and all of the participants were allowed to adjust to the background colours for at least one minute before beginning the test. This minimised the effects of distracting visual stimuli and allowed for adjustment of the subjects' colour vision over time. The viewing distance was 1.4 metres away from the screen, and participants were seated so that they were at eye level with the centre of the screen. The participants were given clear instructions about how the test would be carried out, and they were allowed a test run to make sure they knew what to do. They were provided with a response pad and instructed to press "one of the four red buttons following a 'beep' sound to indicate the direction of motion of the coloured stimulus or the corner towards which the coloured stimulus moved" as shown in Fig. 8 below. The options they were given were lower left (LL), lower right (LR), upper left (UL) and upper right (UR). The apparatus and the results screen for the CAD are also shown in Fig. 6 and Fig. 7 below.

Figure 5: Picture of the CAD test response pad with the four red buttons.

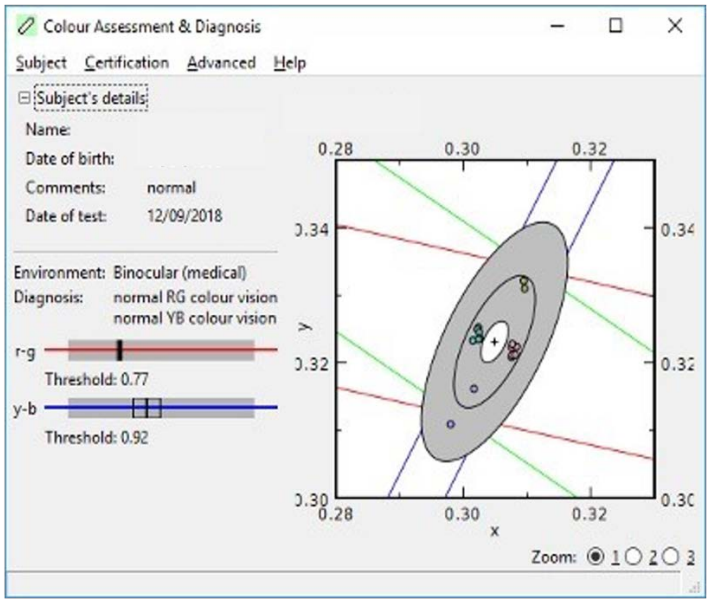

Figure 6: Screenshot of sample results output from CAD test.

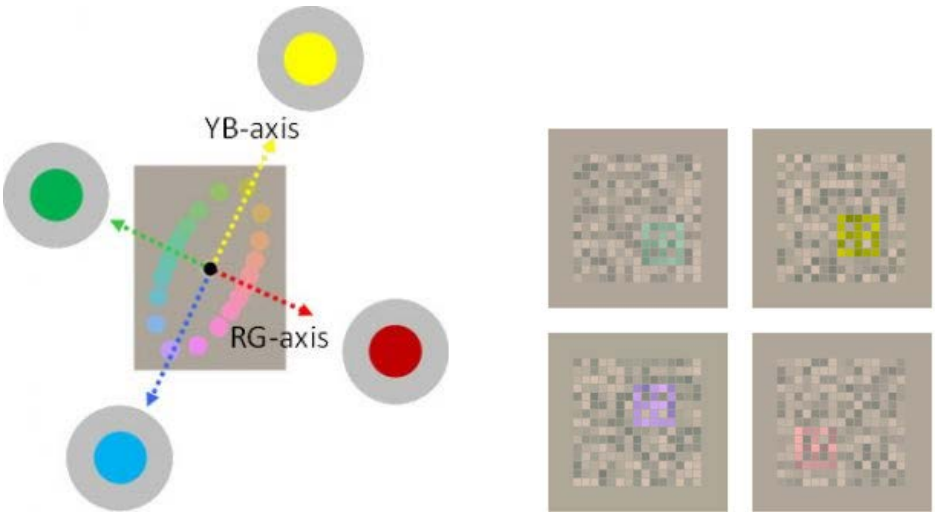

Figure 7: CAD assessment of the YB and RG axes of colour vision. The image on the left demonstrates the axes of interest, and on the right are four presentations corresponding to stimuli that occurred in the RG and YB directions.

3.6.2 Acuity-Plus test

VA testing relies on a limited evaluation of spatial vision, representing the spatial frequency that an individual sees at high contrast. Intermediate and low spatial

frequencies, which are not often checked with standard VA tests, are sometimes the only frequencies affected in patients with diabetic eye disease (Otani et al., 2010). The Acuity-Plus test (AVOT Vision, https://www.city.ac.uk/avot) offers more detailed and accurate measurements for VA and FCS than other methods because it includes a wide range of intermediate and low spatial frequencies. This suggests that this technique may be the most sensitive test available for detecting the changes in acuity associated with diabetes, producing results that are more accurate than the standard Snellen chart used in practice.

In this study, the assessment of VA was important in establishing a baseline measure of the quality of vision for each participant. Spatial vision, under both photopic (daylight) and high mesopic (twilight) conditions, can be affected by changes in the eye's optics or by retinal disease. As light levels decrease, visual performance deteriorates, making everyday tasks, such as reading and face recognition, difficult. This is especially true for older people and those with retinal disease. It has been suggested that more information about the state of the retina is revealed by a person's visual performance in mesopic, rather than photopic, conditions (Katz et al., 2010). This is because mesopic light covers a range of human vision with both rods and cones active. It is a combination of photopic vision and scotopic vision in low-light situations (but not complete darkness). There is no hard-line transition at either end, but the mesopic range is generally considered to be from 3 cd/m^2 down to 0.01 cd/m^2. In the mesopic condition, subjects have larger pupils, leading to increased ocular aberrations and reduced retinal image quality; individuals with early-onset retinal disease often show very large losses of spatial vision in mesopic testing (Katz et al.,

2010). VA may also be affected by diabetic eye disease, which may be indicative of wider problems with visual functioning (Murakami et al., 2011).

FCS provides information on an individual's ability to detect spatial patterns. FCS has been shown to decline with age and is more sensitive to the effects of normal ageing or disease than high-contrast VA. Abnormal FCS is associated with some systemic diseases and ocular defects such as cataracts, diabetic retinopathy, glaucoma, AMD and optic nerve degeneration (Rosenthal & Fischer, 2014). Mesopic contrast sensitivity declines in the 5th decade of a person's life, 10 years before the decline of photopic contrast sensitivity. This could be attributed to the age-related reduction in the number of rods at the parafovea (Gillespie-Gallery et al. (2013)). The Acuity-Plus test for FCS is more sensitive than VA testing, and the results reflect changes in the optics of the eye (such as residual refractive errors, increased scattered light, and large, higher-order aberrations), as well as changes in the retina.

As part of the Acuity-Plus test, stimuli were presented on a high-resolution NEC Multisync Diamondtron CRT monitor, which used a 30-bit colour graphics card with 1280×1024 pixels at a frame rate of 120Hz. The monitor was automatically calibrated with an LMT 1009 luminance meter and the bespoke software that is used to calibrate all AVOT tests (LUMCAL, developed by City Occupational Ltd., London, UK).

The Acuity-Plus test produces a uniform background of constant luminance during the warmup period, after which the specified luminance contrast is reproduced accurately on the stimulus display. Subjects were tested at background luminances of 32.00, 7.60, 3.20, 1.60 and 0.12 cd/m^2. Spectrally calibrated neutral density filters

were used for background luminances below 3 cd/m^2. To examine VA and FCS, participants' correct discrimination of the orientation of the gap in a Landolt C optotype was measured at the fovea and parafovea (5° eccentricity) using a four-alternative, forced-choice procedure for a screen luminance of 32 cd/m^2 for the photopic condition and 0.12 cd/m^2 for the mesopic condition (with spectrally calibrated neutral density filters). Participants viewed the display binocularly from a distance of three metres while sat on a fixed chair. The task was to discriminate the direction of the gap in a Landolt C optotype, which occurred in one of four diagonal directions. Between presentations, a fixation cross and four oblique guides were displayed to help maintain central fixation and accommodation (see Fig. 8). The spectral composition of the background had predominantly long-wavelength and middle-wavelength content to minimise chromatic aberrations and variations in the short-wavelength absorption of light by the macular pigment and the crystalline lens (van de Kraats & van Norren, 2007). The Acuity-Plus test is described in detail by Gillespie-Gallery et al. (2013).

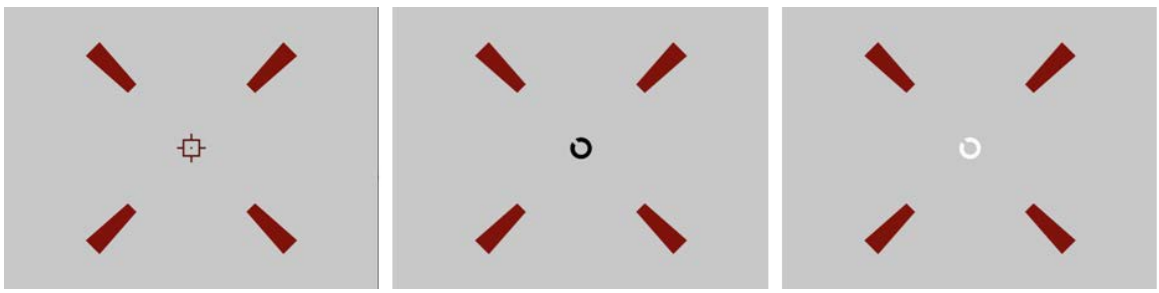

Figure 8: Examples of screen dumps showing the central fixation target, the diagonal guides and the visual stimuli employed in the Acuity-Plus test, with negative (dark Landolt C optotype) and positive (bright Landoldt C optotype) luminance contrast.

VA and FCS were measured using the Landolt C target of 100% contrast defined with either decrements (dark rings) or increments (bright rings) in luminance (see Fig. 9).

The Acuity-Plus test (measuring for both VA and FCS) took around 15–20 minutes to complete for each participant. Therefore, the test could be easily accommodated within a clinical visit alongside the other tests, such as the CAD test (described in section 3.6.1) and the Rod/Cone Sensitivity test (described in the next section). The testing procedure was the same for all participants; the same protocol and test order was adopted for every examination.

Fig. 10 shows an example of a screenshot of the results obtained from an Acuity-Plus test. The results for both photopic and mesopic viewing conditions for positive- and negative-contrast stimuli are shown, including results for both VA and FCS measurements. The number given indicates the subject's actual thresholds (VA is measured in minutes of arc and FCS in percentage luminance contrast).

Figure 9: Screen output from the Acuity-Plus test showing VA and FCS results for both photopic and mesopic binocular measurements, including positive- and negative-contrast sensitivity.

Note: The results show the mean and the ± standard deviation.

3.6.3 Flicker-Plus test

The Flicker-Plus test, a module of the AVOT tests, measures flicker modulation thresholds (FMTs) and provides a measure of the temporal responses of the visual system. Specifically, it measures cone function in photopic vision and rod function under mesopic conditions (see Fig. 11). Normally, the time typically needed for an individual to adapt to the dark is 20–30 minutes. However, with this new, non-invasive test, relatively little dark-adaptation time is required before it is possible to measure FMTs in rod-mediated vision. For the rod protocol, for example, subjects typically need just 1 minute to adapt to the dark before starting the test (we gave them 2–3 minutes to adapt while wearing spectrally calibrated sunglasses). It has been suggested that FMTs can be employed as a functional, photoreceptor-specific

biomarker for the early detection of rod and cone dysfunction due to ageing or pathology such as diabetic retinopathy (Hathibelagal et al., 2020).

The Flicker-Plus test employs several parameters, such as stimulus size, spectral composition, photopic luminance, state of chromatic adaptation and temporal flicker frequency. These are adjusted appropriately to favour either rod- or cone-mediated vision. This test is not sensitive to small, uncorrected refractive errors, higher-order aberrations or moderate levels of scattered light. The thresholds that are measured are, therefore, indicative of the normal functioning of rods and cones and the integrity of the neural retina (Barbur & Rodriguez-Carmona, 2012).

Stimuli were presented either centrally (0° eccentricity) or in four quadrants at 5° eccentricity from a viewing distance of one metre (Fig. 11 and Fig. 12). The five stimulus locations were centre (C), upper left (UL), upper right (UR), lower left (LL), and lower right (LR). The psychophysical method of measuring FMTs was based on a five-alternative, forced-choice procedure designed around the five locations of the stimulus. The stimulus was fixed at 15 Hz for the cone-enhanced condition and 5 Hz for the rod-enhanced condition. Subjects were instructed to maintain their fixation at the centre of the screen to ensure that the peripherally presented stimuli were seen at 5° eccentricity. The subject's task was to detect which region (central or one of the four peripheral fields) that the flicker appeared in. To do so, they used the same response pad as for the CAD test, which had a five-button keypad that mirrored the five locations of the stimulus on the display (see Fig. 6). The location of the flickering stimulus for each presentation was determined at random by the computer program.

Like all the AVOT tests conducted as part of this study, the Flicker-Plus test was run on a desktop computer with two displays: one was used by the researcher to run the tests, and the other generated the visual stimuli for the participant. The two displays were separated by a black curtain so that the participant could only see the stimulus display. The test was conducted on a laptop-driven, 24-inch visual display that had been spectrally calibrated.

The test protocol included a 'learning' phase to ensure that the participant was familiar with the procedure. Each participant was required to achieve 100% correct responses in the learning phase before the full test was administered. For all participants, the cone-enhanced condition was carried out first, after which the participants were given 2–3 minutes to adapt to the light for the rod-enhanced condition by wearing spectrally-calibrated sunglasses (1.0 log unit neutral density filter). This rest period also enabled participants to have a short break between tests. Each test took about six minutes to complete, giving an average of approximately 12 minutes for both tests.

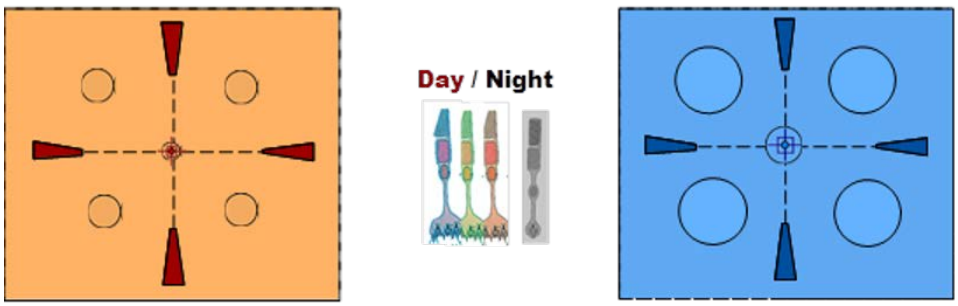

Figure 10: Background display for cone- and rod-enhanced stimulus conditions.

Note: The left panel shows the approximate appearance of the visual display when showing the cone-enhanced stimulus. The stimulus is displayed either at the centre or at an eccentricity of 5° (away from

fixation), diagonally in each of the four quadrants. The real stimulus is a borderless disc, modulated sinusoidally and presented at equal luminance. The right panel illustrates the appearance of the rod-enhanced stimulus condition.

Figure 11: Rod and cone sensitivity test results.

Note: This image provides an overview of the readout of a Flicker-Plus test following the measurement of FMTs. The stimulus locations are numbered on the left and represented on the right from top to bottom in the following order: (1) Centre, (2) Top Left, (3) Top right, (4) Bottom Left and (5) Bottom Right.

Overall, the total testing time for each subject took between 80 and 90 minutes, including the learning phases and any necessary breaks.

3.7 Data and statistical analysis

The previous sections have highlighted the range of tests completed by all the participants in the study. The following sections will explain how these tests were integrated into unified data sets and then analysed using statistical methods. Initially,

a comparison was made between high-risk subjects and diabetic subjects. Subsequently, a more dynamic comparison was conducted across groups (including the control group), and the risk factors associated with colour vision loss in diabetes were assessed.

In the initial stage of the analysis, the parameters measured using the tests defined above were compared across two subject groups: those with pre-diabetes and those with diabetes. The data analysis was intended to accomplish three main goals:

- To examine the type of colour vision loss in subjects already diagnosed with diabetes and confirm the results of previous studies.
- To assess whether RG and YB chromatic sensitivity preceded the onset of diabetes in high-risk subjects.
- To assess VA, FCS, and rod and cone functioning in these subjects.

Firstly, it was necessary to compare the data for each of the two key subject groups as this provided an opportunity to compare the results of the visual tests. This was done through a straightforward comparison of mean test results. This strategy was applied to all the findings relating to VA testing, CAD testing, and the rod and cone sensitivity tests.

As well as comparing the two subject groups directly, intra-group analysis of the data was also conducted. This involved assessing the association between the risk factors for diabetes and the chromatic discrimination thresholds within the same cohort of subjects. This used suitable statistical analysis to assess the data and uncover any

correlation between the variables. A multivariate approach was adopted to ensure that the influence of individual factors could be considered within the context of all the data (controlling for confounding). The following section describes the statistical testing procedures employed.

Throughout the analysis, similar testing protocols were used to compare the findings of high-risk subjects with normal subjects. The analytical process involved intra- and inter-groups analyses. It focused on establishing the significance of any differences in colour vision or general vision (including VA) and correlating these with the risk factors for diabetes. The statistical tests used to facilitate this analysis are also described in the following section, including justifications for the selection of specific tests and consideration of their implications for the outcomes analysed.

3.7.1 Statistical tests

Statistical tests must be selected based on the type of data analysed, as well as the characteristics of that data (i.e., parametric versus non-parametric). This study followed a pragmatic approach, and its primary aim was to discern the relationship between diabetics and high-risk subjects, particularly for colour vision loss.
The initial focus for the statistical analysis of the results was to determine the normality of the data set, based on the distribution of the data according (Kirkwood & Sterne, 2010). The normality of a data set can be influential in determining the assumptions that can be made when engaging in statistical analysis (Bland, 2015). It was necessary, therefore, to examine the normality of all the data sets used in the study. The Kolmogorov–Smirnov test of normality was used to calculate the normality or skewness of each data set. This test is widely used and provides a clear

estimate of normality (Kirkwood & Sterne, 2010). It is worth noting that this test showed that most data sets were not normally distributed (they were positively skewed), except for age in the normal group (subjects without diabetes nor at a high risk of diabetes, see Section 4.3). Parametric and non-parametric tests were chosen according to the findings of the normality test. The key tests that were implemented are detailed below.

All statistical calculations were carried out using SPSS software. The data were not normally distributed as tested ($p < 0.05$, see section 4.3). Therefore, non-parametric statistical package analyses were carried out. The Kruskal–Wallis test was used to evaluate differences between all three groups (subjects with diabetes, those at a high risk of diabetes and normal subjects). Meanwhile, the Mann–Whitney U test (a non-parametric test) was used to compare two specific groups. Initially, high-risk subjects were compared to subjects with diabetes. In this case, the Mann–Whitney U test was used to determine differences in these populations with respect to visual parameters, including RG and YB thresholds. Similarly, high-risk subjects were compared to normal subjects using the Mann–Whitney U test, which assessed any differences between these groups.

Alongside the tests used to compare the three different groups, it was necessary to explore the individual risk factors that might have correlated with higher RG and/or YB CAD thresholds. Therefore, the testing procedures were non-parametric in nature because the skewed data did not fit a normal distribution. As such, a multivariate analysis is recommended in research to provide a clear link between variables and outcomes (Bland, 2015). In this instance, the variables of interest were the risk factors

for diabetes and the outcomes were the RG and YB thresholds in subjects. A suitable multivariate analysis is binary logistic regression analysis, with chi-square as the key outcome, indicative of the effect size of any association between diabetic risk factors and the level of chromatic deterioration. This testing procedure or statistical analysis is widely used in the published studies with similar data sets to those included here (Sokolowska-Oracz & Piatkiewicz, 2017, Lewis et al. 2018, Chaine et al. 1998). Multiple linear regression analysis was also conducted to evaluate the relative effects of specific risk factors while controlling for potential confounding from other risk factors (Petrie & Sabin, 2019).

Two forms of correlation analysis were also conducted as part of the rod and cone sensitivity test. The purpose of the correlation analysis was to compare the findings related to peripheral locations in the groups, whenever this was necessary. For normally distributed data (i.e., subjects in the normal group, Pearson's correlation coefficient was calculated. For the other data sets for the populations showing a positive skew (i.e., the high-risk subject group), Spearman's correlation coefficient was calculated. These coefficients made it possible to use two locations (central and peripheral) rather than five (UL, UR, C, LL and LR) to determine rod and cone sensitivity. The researchers used these two forms of correlation analysis to see if the four peripheral locations of the rods and cones were statistically not different. In this case, the sum of the four peripheral locations was combined into one location (see Section 4.4.2).

For all statistical tests, a suitable level of significance was adopted so that any effects could be attributed to a genuine relationship, rather than mere chance. The standard

p-value of 0.05 was used for this purpose. Effect sizes where $p < 0.05$ are generally thought to be unlikely to occur by chance (Petrie & Sabin, 2019). P-values were reported for all findings where differences in the outcomes between the two populations were noted. A 95% confidence interval is not relevant when using a non-parametric Mann–Whitney U test, as this does not operate using differences in means.

3.8 Rigour in the research process

This section considers the importance of maintaining rigour in the research process described in this chapter. Rigour may be defined as the overall degree to which research adheres to quality standards, ensuring validity and reliability in the methods and the interpretation of the results (Petrie & Sabin, 2019). The key strategies that were used to ensure the validity and reliability of the investigative process are outlined in this section. Also considered are any potential limitations or sources of inherent bias in the study design, methods and tools employed.

Validity refers to the degree to which is measured is accurately and in such a way that the result truly reflects what was intended to be measured (Noble & Smith, 2015). Validity for the present study was particularly important when it came to defining the subject groups. It was necessary to ensure an effective comparison between subjects with a high risk of developing diabetes, subjects with a diabetes diagnosis, and subjects who were not at risk of developing diabetes. It was also important to ensure a valid assessment of chromatic sensitivity and the other visual parameters. Using multiple testing processes in combination made it easier to ensure that the testing process was accurate because the findings could be compared and triangulated. Furthermore, the criteria chosen to stratify and define the subject groups were

reflective of well-established and widely-used criteria for defining the risk factors and clinical markers for diabetes. That said, some measures were self-reported, and not every participant without diabetes underwent a blood test. These could both be considered limitations that may have affected the validity of the different groups into which the subjects were separated.

Reliability refers to the consistency of the findings and the extent to which they are repeatable. Reliability may be achieved in several ways, including through multiple observations of the same phenomenon, adequate data sampling, the use of multiple researchers in analysing data, and the appropriate assessment of consistency in findings (Noble & Smith, 2015). The testing process was considered reliable due to the published reliability of the protocols. There was enough repetition in the data to make it possible to determine mean values. Furthermore, the sample size of the study was sufficient to provide a comparison of mean data across groups, although a sample size large enough to achieve statistical significance was not strictly necessary in this study since each subject's chromatic thresholds were assessed against age-matched upper normal limits. That said, one of the important aims of this study was to establish reliably the percentage of subjects at risk of developing diabetes who show significant colour vision loss and/or rod and cone sensitivity loss. Needless to say, the accuracy of this kind of estimate would increase with sample size. Given the subjects recruited, we remain convinced that the sample size was large enough to allow an accurate estimate of the percentage of subjects at risk of diabetes who exhibit colour vision loss and/or rod and cone sensitivity loss. The optimal sample size was not specifically calculated to avoid minimum data requirements and reflect the feasibility of the method employed. However, given that the study adhered to protocols and

frameworks throughout the analysis, while also ensuring transparency, it is hoped that this will have been sufficient to provide a reliable data set and research method.

4. Results

4.1 Overview

This chapter details the results of the experimental data obtained using the tests and methods discussed in the previous chapter. The final analysis of the data for a total of 213 subjects is presented.

This chapter also describes the characteristics of the population studied, detailing relevant sociodemographic data and dropout rates.

4.2 Subjects

During the initial period of subject recruitment and data analysis, 25 subjects dropped out due to a lack of compliance with the study criteria.

A total of 213 subjects were recruited. 40 subjects had no history of either diabetes or eye disease and were placed in the ‘normal control’ group. 23 subjects had been clinically diagnosed with diabetes, and 150 subjects had been identified as high-risk candidates for diabetes. Subjects were divided into these groups based on the inclusion and exclusion criteria outlined in Section 3.4. The analysis focused on subjects across all the groups.

4.3 Normality

The data were checked for normal distribution using IBM SPSS Statistics for Windows, Version 25. Normality was tested using both the Kolmogorov–Smirnov test and the Shapiro–Wilk test. These tests show whether the given quantitative

variable (in this case, the CAD RG threshold or photopic FCS) is normally distributed or not normally distributed. This is one of the most important assumptions when it comes to parametric tests. The results of this test are shown in Appendix E.1.

All the visual parameters in this study were not-normally distributed, hence violating the assumptions made in some parametric testing. In response, non-parametric tests were employed where these parameters were considered as dependent variables to enable intra- and inter- group comparisons.

The lack of normal distribution (summarised in Appendix E.2) led to the use of the Kruskal–Wallis test for global significance testing (see the next section) to compare all three groups.

4.3.1 Global significance testing

To test the global significance between the two groups, a Mann–Whitney U test was used, and a Kruskal–Wallis test was used to test the significance for more than two groups. The overall results of the test suggested that there were statistically significant differences between the groups, validating the group stratification and the process of differentiation. These differences were evident for every variable relating to visual function, suggesting that the groups showed key differences in overall (or global) visual functioning and outcomes. This finding lends support to the notion that diabetes and high-risk subjects have specific visual deficits compared to the general, healthy population. It also partially validated previous studies that demonstrated that there is a spectrum of visual deficits among diabetics at different stages of the disease process. Importantly, this global analysis validated the use of the control population

as they had distinct features to the diabetic subjects, as would be expected in a random sample of the healthy population.

Following the global significance test, each pair of groups was compared separately. The key visual variables, mentioned in the previous chapter, were all assessed. The remainder of this chapter documents the findings for the visual tests for the paired groups, as well as the risk factors associated with visual deficits across groups.

As shown by Table 3, the comparisons of the groups were significantly different for all the parameters that were measured. A statistically significant overall difference was found across all groups (see Appendix E.2). Table 3 also shows that all the visual parameters were significantly different between the three subject groups.

Following the global significance test, each pair of groups was compared separately. The key visual variables, mentioned in the previous chapter, were all assessed. The remainder of this chapter documents the findings for the visual tests for the paired groups, as well as the risk factors associated with visual deficits across groups.

Table 3: Global significance across all groups.

Visual parameters	High risk (n = 150)	Normal (n = 40)	Diabetics (n = 23)	Kruskal–Wallis test		
	Mean ± SE	Mean ± SE	Mean ± SE	Chi Square	df	P-value
photopic VA (+)	1.1 ± 0.03	0.9 ± 0.03	2.2 ± 0.42	20.048	2	.000, Sig
photopic VA (-)	1.0 ± 0.04	0.8 ± 0.02	2.1 ± 0.48	21.846	2	.000, Sig
photopic FCS (+)	12.8 ± 0.62	9.9 ± 0.52	53.9 ± 21.4	19.197	2	.000, Sig
photopic FCS (-)	11.1 ± 0.64	8.3 ± 0.77	32.1 ± 6.85	28.440	2	.000, Sig
mesopic VA (+)	2.0 ± 0.04	1.8 ± 0.06	3.5 ± 0.49	25.809	2	.000, Sig
mesopic VA (-)	1.8 ± 0.05	1.6 ± 0.05	3.2 ± 0.47	14.932	2	.001, Sig
mesopic FCS (+)	50.0 ± 1.7	45.7 ± 2.1	105.3 ± 24.24	9.664	2	.008, Sig
mesopic FCS (-)	45.7 ± 1.52	40.3 ± 1.71	61.7 ± 6.15	7.553	2	.023, Sig
CAD RG	1.9 ± 0.15	0.9 ± 0.04	4.1 ± 1.53	56.462	2	.000, Sig
CAD YB	2.2 ± 0.16	1.0 ± 0.04	5.1 ± 0.97	71.390	2	.000, Sig
cone vision LL	2.9 ± 0.10	1.8 ± 0.06	6.33 ± 0.6	64.573	2	.000, Sig
cone vision LR	2.7 ± 0.1	1.6 ± 0.07	6.38 ± 0.81	77.721	2	.000, Sig
cone vision C	3.8 ± 0.18	2.0 ± 0.1	17.7 ± 4.56	79.901	2	.000, Sig
cone vision UR	2.8 ± 0.11	1.7 ± 0.07	6.0 ± 1.21	52.301	2	.000, Sig
cone vision UL	2.9 ± 0.11	1.9 ± 0.08	6.0 ± 0.81	61.713	2	.000, Sig
rod vision LL	4.5 ± 0.17	2.7 ± 0.08	8.5 ± 1.27	54.810	2	.000, Sig
rod vision LR	4.0 ± 0.14	2.4 ± 0.12	8.5 ± 1.27	62.426	2	.000, Sig
rod vision C	6.8 ± 0.27	3.5 ± 0.23	16.9 ± 4.41	43.710	2	.000, Sig
rod vision UR	4.2 ± 0.15	2.5 ± 0.11	8.8 ± 1.84	60.820	2	.000, Sig
rod vision UL	4.2 ± 0.14	2.5 ± 0.08	8.2 ± 113	68.219	2	.000, Sig

Note: (-) denotes negative contrast stimuli; (+) denotes positive contrast stimuli.

4.4 Assessment of visual performance

This section reports the results obtained from several of the tests described in Section 3.6, each of which measured different aspects of visual performance.

4.4.1 Chromatic sensitivity

The CAD test (see Section 3.6.1) was employed to assess RG and YB chromatic sensitivity in all subject groups.

Table 4 shows that there were significant differences in the RG and YB CAD thresholds between the high-risk and diabetic subject groups. Based on the analysis of 173 subjects (150 with a high risk of diabetes and 23 with diabetes) the mean (± standard error) for the RG threshold was 4.05 ± 1.53 and 1.86 ± 1.15 for the diabetic and high-risk groups, respectively. This difference was found to be statistically significant ($p = 0.01$). This was also the case for the YB threshold ($p < 0.0001$), where means of 5.10 ± 0.97 and 2.18 ± 0.16 were obtained for subjects with diabetes and subjects at a high risk of diabetes, respectively.

Table 4: Mean RG and YB colour thresholds on CAD testing in high-risk and diabetic groups.

CAD test	Diabetic (n = 23)	High-risk group (n = 150)	Mann–Whitney U test	P-value
Mean RG ± SE	4.05 ± 1.53	1.86 ± 1.15	977.50	0.01, Sig
Mean YB ± SE	5.10 ± 0.97	2.18 ± 0.16	581.00	<0.0001, Sig

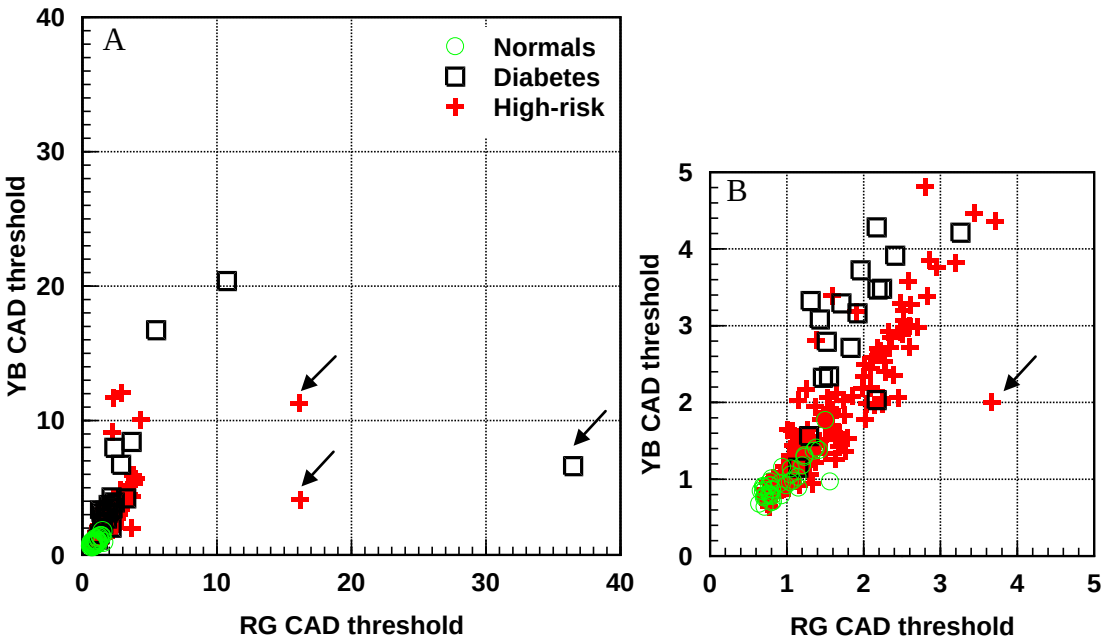

Figure 12: RG versus YB CAD thresholds plotted for all subject groups.

Note: Normal subjects are designated by green circles, patients with diabetes by black squares, and subjects at a high risk of developing diabetes by red crosses. (A) shows all subject groups. (B) shows an expanded version of the plot up to 5 CAD units.

Fig. 13 shows a plot of RG CAD threshold versus YB CAD threshold for all the subject groups examined. Four subjects, one with diabetes and three with a high risk of diabetes, were identified as having a congenital colour vision deficiency because they had higher RG thresholds than YB thresholds (see Table 5). Therefore, they were excluded from the study. Of these four subjects, three also had abnormal YB thresholds, indicative of acquired colour loss. As can be seen from Fig. 13 (B), subjects in the normal group are clustered around 1 unit for RG and 1 unit for YB, as expected (see Table 7 for results in the normal subject group).

Table 5: RG and YB thresholds of the four subjects identified with congenital colour deficiency (three from the high-risk group and one from the diabetic group).

Subjects with CCD	CAD RG	CAD YB
Diabetic (n = 1)	36.48	6.61
High risk (n = 3)	3.98 16.40 16.18	1.99 11.51 4.13

The four subjects were not aware of their congenital colour deficiency. It was useful to see that the CAD test was able to differentiate acquired loss from congenital loss.

Fig. 14 reveals the differences in RG and YB thresholds within the high-risk and diabetic subject groups.

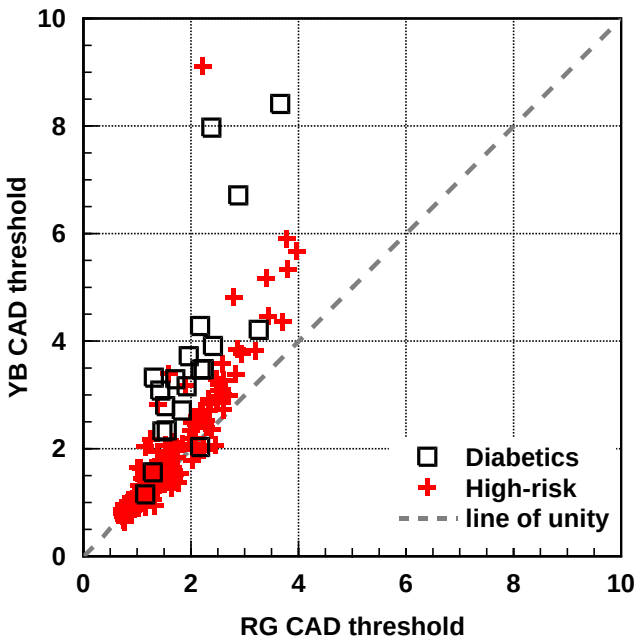

Figure 13: Comparative analysis of RG versus YB CAD threshold data for the diabetic and high-risk groups.

Note: The line of unity is shown, which makes it possible to assess whether YB or RG thresholds are more commonly affected.

The results obtained in the groups shown in Fig. 14 show greater YB loss compared to RG loss. The line of unity has been added to the graph to demonstrate this visually. These findings support those of other studies, which also reported greater levels of YB loss (O'Neill-Biba et al., 2010; Barbur et al., 2012). It is also worth noting that when the thresholds were less than ~2 CAD units, the loss of RG and YB was more balanced, but when the loss of chromatic sensitivity became more pronounced, the YB threshold was typically affected more than the RG threshold.

Figs. 15 and 16 plot the same data as Figs. 13 and 14 while also accounting for normal ageing. Colour vision is affected by natural ageing (Barbur & Rodriguez-Carmona, 2015). Hence, these results show whether there are other factors as well as ageing that influence the loss of colour vision, such as the risk factors for diabetes. Fig. 15 shows how RG chromatic sensitivity is affected by normal ageing. For a more detailed description of this, see Barbur and Rodriguez-Carmona (2015). The results for the high-risk and diabetic groups have been superimposed on top of the normal age-dependent limits for RG (Fig. 15) and YB (Fig. 16) thresholds. The black dotted line represents the median normal threshold for a specified age, and the red dotted lines above and below correspond to the ± 2.5 standard deviation limits. The results show that both subject groups display RG thresholds above the median. This is more evident in the lower graph in each figure, which shows the data at an expanded scale. If one had a normally distributed population, one would expect an equal distribution of values around the median, but this was not the case for either group in Fig. 15. Overall, the RG thresholds were skewed above the expected median values of the normal population. In addition, no subjects in the diabetic group had RG thresholds below the median, and 41% had thresholds above the upper limit (2.5 SD).

Surprisingly, in the high-risk group, 32% of subjects had RG thresholds above the upper limit.

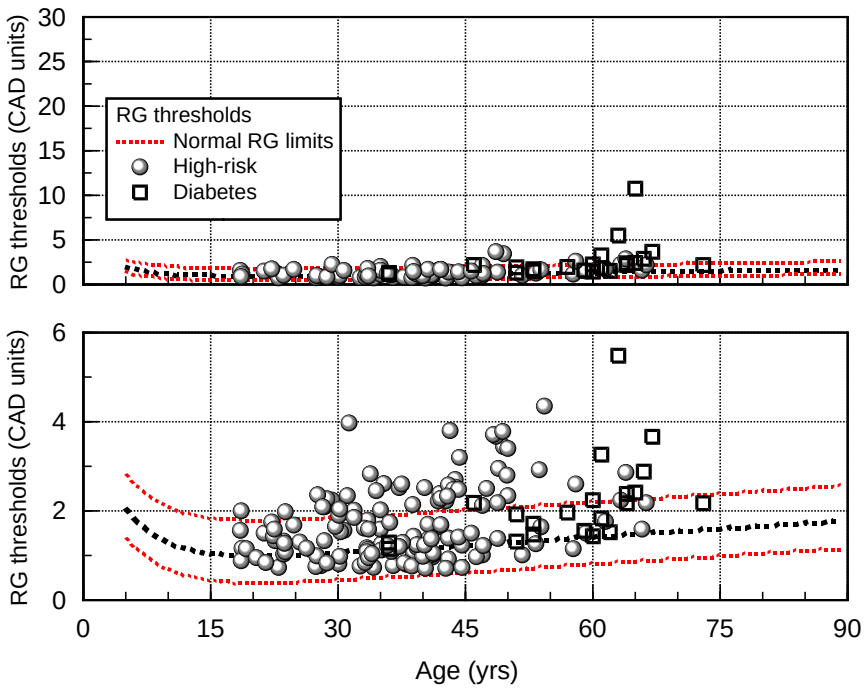

Figure 14: RG thresholds for the high-risk and the diabetic groups plotted against age (the graph was adapted from Barbur & Rodriguez-Carmona, 2015).

Note: The median values (black dotted line) and ±2.5σ limits (red dotted lines) are shown for normal trichromats. The results show that 41% of the diabetic group have RG thresholds above the upper 2.5σ limit. No diabetic subjects have RG thresholds below the median threshold for their age. Surprisingly, the high-risk group have 32% of RG thresholds above the 2.5σ limit.

Similar results were noted when YB thresholds were analysed (see Fig. 16). Subjects at a high risk of developing diabetes tended to have thresholds close to or above the median value, while all subjects in the diabetic group had thresholds that exceeded the median normal value. Overall, the YB thresholds were skewed above the expected median values of the normal population. 40% of the high-risk subjects had thresholds above the upper +2.5 SD limit. None of the diabetic group had YB

thresholds below the median; 86% had thresholds above that upper limit (Fig. 16). This pattern suggests that the median normal threshold value was exceeded in almost all subjects in both the high-risk and diabetic groups.

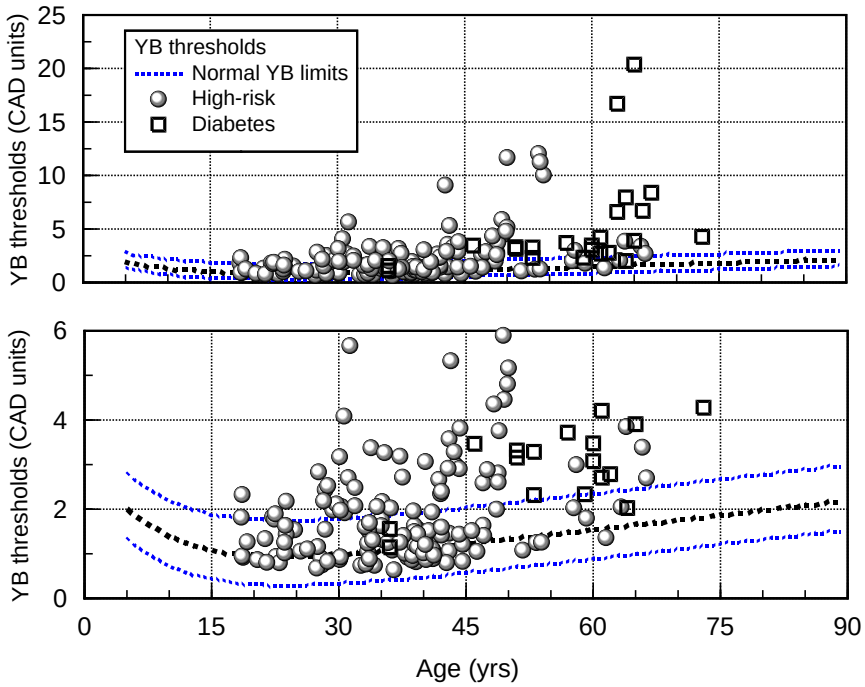

Figure 15: YB-axis threshold data for the high-risk and the diabetic groups versus age (the graph was adapted from Barbur & Rodriguez-Carmona, 2015).

Note: The top figure shows enlarged axes, and the bottom graph is an expanded scale. The median YB CAD thresholds for a normal population (black dotted line) and the corresponding ±2.5σ limits (blue dotted line) are shown. The results show that 41% of the diabetic group have RG thresholds above the upper 2.5σ limit, and 86% of the diabetic group have YB thresholds above the upper limit. No subjects had either RG or YB thresholds below the median threshold for their age. Surprisingly, the high-risk group had 32% RG thresholds and 40% YB thresholds above the 2.5σ limit.

Overall, including both the RG and YB thresholds, over 80% of the diabetic subjects examined exhibited colour thresholds above the upper limits for age-matched normal subjects. These findings were higher than those previously reported by Barbur et al. (2012). The remaining 20% had colour thresholds within the normal range but above the median for age-matched normal subjects. Also, it is worth noting that over 40% of

the subjects in the high-risk group had RG and YB colour thresholds above the upper limit for age-matched normal subjects. This suggests that diabetes and pre-diabetes disease processes affect chromatic sensitivity, leading to a greater colour vision loss than would be anticipated in normal ageing.

Table 6 details the differences in mean RG and YB CAD thresholds between the high-risk and normal groups. The results show that both the RG and YB thresholds were significantly higher in the high-risk group compared to the normal group. These results are displayed visually in Fig. 17, which shows the relative balance of RG and YB colour thresholds. These results demonstrate that the normal subjects examined in Kuwait were no different from the normal subjects examined at City, University of London by Rodriguez-Carmona et al. (2005). This is important because the City, University of London data set forms the basis for the Standard CAD observer. In the present study, the CAD thresholds ranged from 0.64 to 1.56 for RG and 0.64 to 1.77 for YB. The age range of the normal group in this study was from 19 to 53 years of age.

Table 6: RG and YB thresholds compared in high-risk and normal subject groups.

CAD test	Normal (n = 40)	High-risk group (n = 150)	U value	P-value
RG (Mean ± SE)	0.94 ± 0.04	1.86 ± 1.15	1010.50	< 0.001, Sig
YB (Mean ± SE)	0.97 ± 0.04	2.18 ± 0.16	959.50	< 0.001, Sig

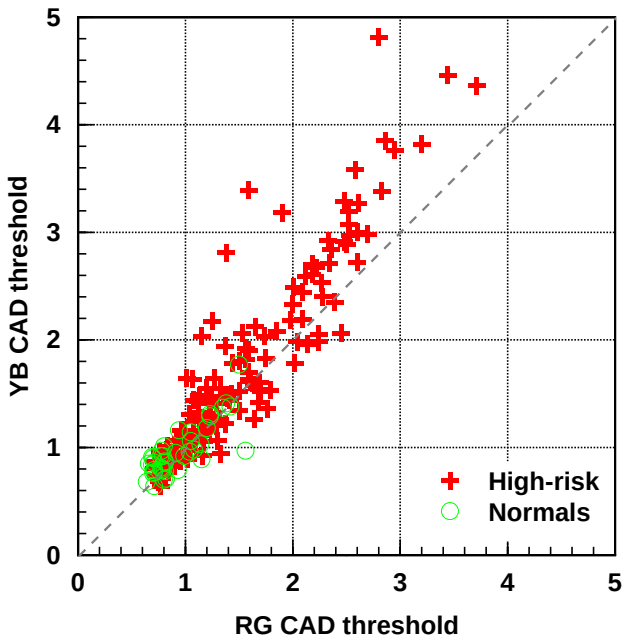

Figure 16: Comparative analysis of RG versus YB CAD threshold data for the normal and high-risk groups.

Note: The line of unity is shown, which makes it possible to assess whether YB or RG thresholds are most affected.

In summary, the analysis of YB- and RG-axis loss shows that in subjects with diabetes there is a pronounced level of colour vision loss. This is particularly skewed towards deficits in the YB thresholds, although RG chromatic sensitivity is also affected. Subjects at a high risk of diabetes show a more diffuse pattern of loss, affecting both the YB and RG axes up to a measure of two CAD units. This then shifts towards a greater YB loss, although the patterns of chromatic sensitivity loss are not massively dissimilar to those observed in subjects with diabetes. This pattern suggests that colour loss is linked to the diabetes disease process, independent of the natural ageing of the human colour system.

4.4.2 Rod and cone sensitivity

Rod and cone sensitivity were examined using the Flicker-Plus test, which measures the spectral, spatial and temporal properties of vision mediated by rods and cones. This test provides a measurement at one central and four peripheral locations. Elevations of the thresholds for rod and cone sensitivity were analysed graphically (see Figs. 18 and 19), as well as statistically, to appreciate how sensitivity was affected across different stimulus locations. The findings highlight similar patterns of sensitivity in all subject groups, with higher thresholds noted in the Binocular Cone Vision centre location (0°), (BCV) (0°), compared to other locations. This shows that there was a homogeneous shift in threshold values for all the stimulus locations in the target group (high-risk subjects), compared to the normal group, suggesting a more uniform disease process and loss of rod and cone sensitivity. Although the shift was largely homogeneous, as noted above, there was some evidence that the central stimulus location showed a greater level of threshold increases than the other regions. Similar results were noted when comparing the data on rod sensitivity for high-risk and normal subjects. The increase in the threshold value at the centre location, BCV (0°), was greater than that observed at the other locations.

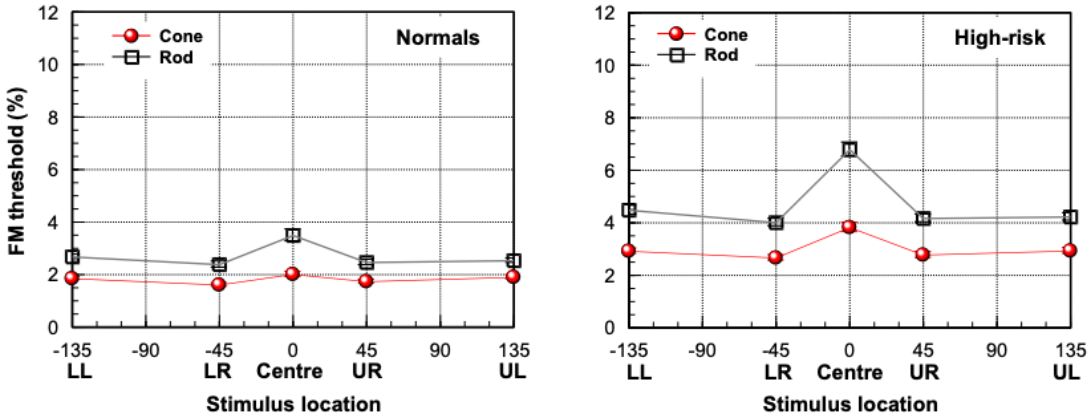

Figure 17: Flicker modulation thresholds in rods and cones in normal and high-risk subjects.

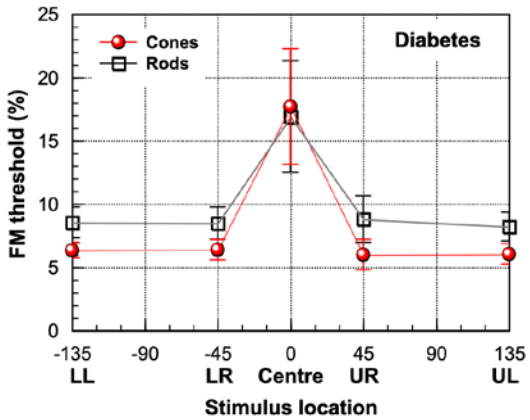

Figure 18: Flicker modulation thresholds in rods and cones in diabetic subject groups.

Flicker modulation thresholds for the diabetic group are shown in Fig. 19. The results reveal that flicker modulation was greatly affected in the diabetic subjects. This suggests that there was more flicker sensitivity loss for subjects whose diabetes was at a later stage of development. It was less present in the high-risk population.

Table 7 shows a statistical significance in both rod and cone sensitivity for all the eccentricities between the high-risk and diabetic groups (n = 173). This suggests that

there is probably a gradient of sensitivity loss as diabetes progresses, even in subjects without a clinical diagnosis who are at risk of developing the disease.

Table 7: Rod and cone sensitivity differences in the high-risk and diabetic subject groups.

		Diabetic (n = 23)	High-risk group (n = 150)	Mann–Whitney U test	P-value	Sig
Cone (Mean±SE)	LL	6.33 ± 0.60	2.91 ± 0.10	332.50	< 0.0001	Sig
	LR	6.38 ± 0.81	2.66 ± 0.10	331.00	<0.0001	Sig
	C	17.68 ± 4.56	3.82 ± 0.16	306.00	<0.0001	Sig
	UR	5.99 ± 1.21	2.77 ± 0.11	653.50	<0.0001	Sig
	UL	6.03 ± 0.81	2.92 ± 0.11	525.00	<0.0001	Sig
Rod (Mean±SE)	LL	8.53 ± 1.27	4.47 ± 0.17	672.00	<0.0001	Sig
	LR	8.47 ± 1.27	4.00 ± 0.14	591.00	<0.0001	Sig
	C	16.89 ± 4.41	6.78 ± 0.27	937.50	<0.0001	Sig
	UR	8.80 ± 1.84	4.16 ± 0.15	690.50	<0.0001	Sig
	UL	8.20 ± 1.13	4.22 ± 0.14	774.50	<0.0001	Sig

In both groups, rod and cone sensitivity thresholds were above those found in normal subjects. In addition, the results from the diabetic group show that rod and cone sensitivity was particularly affected in the central foveal location.

Table 8 provides a comparison between the normal group and the high-risk group. It reveals significant differences in cone and rod sensitivity across all the locations analysed. Overall, the analysis confirmed that there was a statistically significant reduction in rod and cone sensitivity in high-risk subjects compared to the normal group ($p < 0.001$ for all parameters).

Table 8: Rod and cone sensitivity comparison between the high-risk and normal subject groups.

		Normal (n = 40)	High-risk group (n = 150)	U value	P-value	Sig
Cone (Mean±SE)	LL	1.84 ± 0.06	2.91 ± 0.10	1264.00	<0.001	Sig
	LR	1.60 ± 0.07	2.66 ± 0.10	1152.00	<0.001	Sig
	C	2.00 ± 0.10	3.82 ± 0.16	1007.50	<0.001	Sig
	UR	1.73 ± 0.07	2.77 ± 0.11	1489.00	<0.001	Sig
	UL	1.89 ± 0.08	2.92 ± 0.11	1368.50	<0.001	Sig
Rod (Mean ±SE)	LL	2.67 ± 0.08	4.47 ± 0.17	1252.50	<0.001	Sig
	LR	2.34 ± 0.12	4.00 ± 0.14	1134.50	<0.001	Sig
	C	3.49 ± 0.23	6.78 ± 0.27	1048.50	<0.001	Sig
	UR	2.46 ± 0.11	4.16 ± 0.15	1260.00	<0.001	Sig
	UL	2.53 ± 0.08	4.22 ± 0.14	1066.50	<0.001	Sig

In the high-risk and diabetic subject groups (positively skewed), Spearman's correlation was used. In the normal subject group (normally distributed), the Pearson correlation was used.

To make it easier to approach the analysis, the differences between the four peripheral locations were examined using Pearson and Spearman's correlation tests for the three subject groups. Interestingly, statistically significant differences were not observed in any of these four locations among the normal, high-risk and diabetic groups. This implied that the four peripheral measurements could be averaged, and the results could be reported as a single peripheral measurement (see Table 9). This is a simplification and is suitable for reporting rod and cone sensitivity loss in subjects

who are likely to have uniform loss across all four locations (that is, subjects with relatively mild loss).

Table 9: Averaged peripheral and central measurement for the rod and cone sensitivities of each of the three subject groups.

		Diabetic (n=23)	High-risk group (n=150)	Normals (n=40)	P-value	Sig
Cone (Mean±SE)	C	17.68 ± 4.56	3.82 ± 0.16	2.00 ± 0.10	<0.0001	Sig
	Per	6.18 ± 0.81	2.82 ± 0.10	1.77 ± 0.06	<0.0001	Sig
Rod (Mean±SE)	C	16.89 ± 4.41	6.78 ± 0.27	3.49 ± 0.23	<0.0001	Sig
	Per	8.50 ± 1.24	4.21 ± 0.15	2.51 ± 0.09	<0.0001	Sig

Note: There is a statistical difference between all three groups for both the central location (C) and the peripheral location (Per).

The four peripheral locations in the normal and high-risk subjects show small standard errors. This is probably due to the lack of retinal abnormalities. Despite there being no statistical significance in the four peripheral measurements among the diabetic group, the standard error was larger. The peripheral uniformity is likely to break down in the early stage of diabetic retinopathy or in other retinal conditions where the localised rod and cone sensitivity measurements would be recommended.

The following section considers the effect of diabetes on VA and FCS, both of which are important markers of overall visual functioning in adults. This section complements the data on chromatic sensitivity loss, providing a basis for analysing

the specific forms of colour loss and visual defects that are seen in high-risk and diabetic subjects.

4.4.3 Visual acuity

The Acuity-Plus test offers optimised tests of VA and FCS. It is a measure of contrast sensitivity that is easy to use and is relevant in occupational environments. Contrast sensitivity is an important way of determining visual functioning at different spatial frequencies and different luminances. Both VA and FCS are used to calculate the overall capacity of an individual's visual system.

The final test carried out was the assessment of VA and FCS within the Acuity-Plus testing protocol. This section focuses specifically on VA to provide a general overview of visual functioning and insights into any specific deficits in VA that may emerge within the context of the development of diabetes.

To examine the VA results of the three groups, a Kruskal–Wallis test was applied using SPSS. Two comparisons are presented within the final data set: the first compares the diabetic group with the high-risk group; the second compares the normal group with the high-risk group. The initial comparison of the high-risk and diabetic groups showed statistically significant differences in VA for all parameters, with both positive- and negative-contrast VA under photopic and mesopic conditions. The results are presented in Table 10. The high-risk group have normal (~6/6 - 6/12) VA under both photopic and mesopic conditions. The diabetic group, however, showed losses greater than ~6/12 for VA under photopic and mesopic conditions. This suggests that subjects with a high risk of diabetes experienced less disruption of

their VA compared to subjects with established diabetes (at a statistically significant level).

Table 10: VA assessment in diabetic and high-risk subjects.

		Diabetic (n = 23)	High risk (n = 150)	Mann–Whitney U test	P-value, Sig
Photopic (Mean±SE)	VA	2.2 ± 2.0 (+)	1.08 ± 0.03 (+)	883.50	<0.0001, Sig
		2.11± 2.3 (-)	1.03 ± 0.04 (-)	903.50	<0.0001, Sig
Mesopic (Mean±SE)	VA	3.5 ± 2.3 (+)	1.97 ± 0.04 (+)	800.50	<0.0001, Sig
		3.2 ± 2.2 (-)	1.80 ± 0.05 (-)	983.00	0.001, Sig

Note: (-) denotes negative contrast stimuli; (+) denotes positive contrast stimuli.

Table 11: VA assessment in high-risk and normal subject groups.

		Normal (n = 40)	High-risk group (n = 150)	Mann–Whitney U test	P-value, Sig
Photopic (Mean±SE)	VA	0.93 ± 0.03 (+)	1.08 ± 0.03 (+)	2441.50	0.07, NS
		0.83 ± 0.02 (-)	1.03 ± 0.04 (-)	2352.00	0.03, Sig.
Mesopic (Mean±SE)	VA	1.75 ± 0.06 (+)	1.97 ± 0.04 (+)	2207.50	0.01, Sig
		1.62 ± 0.05 (-)	1.80 ± 0.05 (-)	2581.50	0.17, NS

Note: (-) denotes negative contrast stimuli; (+) denotes positive contrast stimuli.

Table 11 presents a comparison of the normal and high-risk groups. As visual parameters across these groups were not normally distributed, a Mann–Whitney U test was conducted to ensure non-parametric testing of the VA parameters in each group. Unlike the comparison between diabetics and high-risk subjects, the significance between the normal and high-risk groups was only noted in negative-contrast VA under photopic conditions and in positive-contrast VA under mesopic conditions (p = 0.03

and 0.01, respectively). The findings for positive-contrast VA (photopic conditions) and negative-contrast VA (mesopic conditions) were not statistically significant between the two groups (see Table 11). Thus, the data was not conclusive, but it did suggest that some changes in VA were taking place in the high-risk subjects.

In summary, these findings suggest that VA measures may provide some indication of the early signs of diabetes, but the findings are inconclusive. Nevertheless, the findings do reveal that people tend to experience a gradual loss in VA as diabetes develops.

4.4.4 Functional contrast sensitivity

FCS is a key marker of spatial defects in contrast sensitivity and is reflective of visual functioning and quality of vision. FCS is a measure of how much a pattern must vary in contrast to be seen, whereas VA measures how big an object must be to be seen. The test was conducted in the manner previously described in Section 3.6.2, in both photopic and mesopic conditions. FCS is an important measurement that complements the measurement of VA.

Table 12 shows a comparison of the FCS findings for the diabetic and high-risk groups. A statistically significant difference was found for both photopic and mesopic conditions and for both positive- and negative-stimulus contrasts. These results are similar to the findings shown in Table 13, which compares diabetics and high-risk subjects. Although all four conditions show some significance, the results suggest that VA is a slightly more sensitive measure than FCS. Again, these results are

indicative of the progressive nature of the diabetes disease process, even without the presence of diabetic retinopathy.

Table 12: FCS assessment in diabetic and high-risk subjects.

		Diabetic (n = 23)	High risk (n = 150)	Mann–Whitney U test	P-value, Sig
Photopic	FCS	53.9 ± 102.6 (+)	12.75±0.62 (+)	926.00	<0.0001, Sig
		32.1 ± 32.8 (-)	11.07 ± 0.64 (-)	730.50	<0.0001, Sig
Mesopic	FCS	105.3 ± 116.3 (+)	49.96 ± 1.70 (+)	1070.50	0.003, Sig
		61.7 ± 29.5 (-)	45.68 ± 1.52 (-)	1211.50	0.02, Sig

Note: (-) denotes negative contrast stimuli; (+) denotes positive contrast stimuli.

Table 13: FCS assessment in high-risk and normal subject groups.

		Normal (n = 40)	High-risk group (n = 150)	Mann–Whitney U test	P-value, Sig
Photopic	FCS	9.91 ± 0.52 (+)	12.75 ± 0.62 (+)	2394.50	0.05, Sig.
		8.31 ± 0.77(-)	11.07 ± 0.64(-)	2288.00	0.02, Sig
Mesopic	FCS	45.69 ± 2.10 (+)	49.96 ± 1.70(+)	2817.00	0.55, NS
		40.34 ± 1.71(-)	45.68 ± 1.52(-)	2590.50	0.19, NS

Note: (-) denotes negative contrast stimuli; (+) denotes positive contrast stimuli.

Table 13 provides a comparison between the high-risk group and the normal group. Interestingly, the FCS comparison between the high-risk and normal groups did show some level of significant difference, especially under photopic conditions for both positive and negative contrast ($p = 0.05$ and $p = 0.02$, respectively). FCS was not statistically different in the mesopic condition for either positive- or negative-contrast stimuli ($p > 0.05$).

It is worth pointing out that VA and FCS (measured using the Acuity-Plus protocol) reveal adverse effects in subjects in the early stages of the diabetic disease process. Unexpectedly, though, mesopic FCS was shown to be similar between normal subjects and high-risk subjects, indicating that the risk factors and pathology for early diabetes may have a less pronounced effect on mesopic FCS than on mesopic VA.

4.4.5 Summary of visual performance testing

All subjects underwent the visual performance tests independently (including CAD, rod and cone sensitivity, and Acuity-Plus assessments). When comparing subjects with diabetes with those at a high risk of diabetes, it was notable that statistical differences between the two groups were found for all measures of visual performance. In terms of chromatic sensitivity, the findings reveal a more pronounced level of loss in subjects with diabetes. Moreover, clinical diabetes generally affects the YB thresholds to a greater extent. The diabetic group also showed worse rod and cone sensitivity, especially at the central location (0°). VA and FCS were also significantly affected in the diabetic group.

When it comes to the differences between the high-risk and normal groups, it is worth noting that the normal group recruited in Kuwait had similar CAD thresholds and VA to those measured routinely at City, University of London. In terms of chromatic sensitivity, the high-risk group had elevated RG and YB thresholds compared to the normal group. These results reveal that the loss was more equal in nature for milder losses. When the losses were greater than ~2 CAD units, YB loss became predominant. The findings also show that the high-risk group experienced a

deterioration in VA and FCS compared to the normal group, although the findings were less consistent. These findings suggest that chromatic sensitivity is the most robust indicator of visual performance changes and that it is much more sensitive than VA or FCS as a measure in early-stage diabetes.

The following section builds on these findings to explore which of the risk factors associated with diabetes cause greater losses in chromatic sensitivity, rod and cone sensitivity, and VA and FCS sensitivity.

4.5 Analysis of diabetic risk factors

The final part of the data-collection process involved identifying and grading the diabetic risk factors that led to greater losses in visual function. Table 15 provides the mean age and BMI for the three subjects groups, and the percentage of subjects in each group who have that condition or risk factor. A multiple linear regression model is used to investigate the link between the diabetic risk factors and visual performance parameters.

From Table 14, it is clear that the mean age was higher in subjects with a diagnosis of diabetes compared to those at a high risk of diabetes (58.08 versus 38.12 years). The mean BMI, however, was almost the same in both groups, while high fasting blood glucose level was higher in the high-risk group than the diabetic group. Having a history of hypertension was more common in subjects with diabetes than those at a high risk of diabetes (43.5% compared to 22.0%).

Table 14: Risk factors for diabetes in all three groups.

Risk factors	Normal (n = 40)	High-Risk (n = 150)	Diabetic (n = 23)
Mean age (years ± SE)	37.2 ± 1.54	38.12 ± 0.87	58.08 ± 1.93
Mean BMI (body mass index ± SE)	23.25 ± 0.178	25.26 ± 0.129	25.65 ± 2.44
Family history of diabetes (%)	0	78.6	100
Smoking (%)	0	34.7	17.40
Unhealthy diet (%)	47.5	69.3	56.5
Hypertension (%)	0	22.0	43.5
No Exercise (%)	0	48.7	52.2
High Fasting Blood glucose (%)	0	46.0	30.4
High HbA1C (%)	-	-	60.9

Analysing the normality of the high-risk and diabetic groups revealed that the data were not normally distributed (See appendix E.1). Therefore, a non-parametric test procedure was used to compare risk factors across the groups, and a chi-square test was used to explore the association between the groups. The results of the chi-square analysis are shown in Table 15. The only statistically significant differences were those observed for the following risk factors: age ($p < 0.001$), family history ($p < 0.01$) and hypertension ($p<0.03$). These results are not unexpected, as advanced age is associated with the development of diabetes (as well as with the transition from pre-diabetes to diabetes), hypertension is often associated with diabetes; it may be at an intermediate level of frequency in high-risk subjects and family history is also a strong predictor of diabetes. Unfortunately, HbA1c could not be compared as it is not part of the routine assessment carried out in pre-diabetics.

Table 15: Association between risk factors and group status. A chi-square test was used to explore the association between diabetic and high-risk groups across all risk factors.

Risk factors	Chi square	P-value, sig
Age	333.00[a]	<.0001, Sig
BMI	1715.50[a]	0.97 NS
Family history of diabetes (Yes/No)	6.02	0.01 Sig
Fasting blood glucose (Yes/No)	1.96	0.16 NS
Smoking (Yes/No)	2.72	0.10 NS
Unhealthy diet (Yes/No)	1.50	0.22 NS
Hypertension (Yes/No)	4.93	0.03 Sig
Exercise (No/Rarely/Twice a week/ Daily)	2.14	0.60* NS

[a] Mann-Whitney U value

***** Fisher's exact p-value.

A regression analysis of risk factors was performed to determine the association between risk factors identified in high-risk subjects and correlate these with the losses of chromatic sensitivity and rod and cone sensitivity. As a predictive analysis, multiple linear regression is used to explain the relationship between one continuous dependent variable and two or more independent variables, whether continuous or categorical.

In this case, the regression analysis assessed the relationship between the continuous dependent variable (RG CAD, YB CAD, and rod and cone sensitivity at the centre and the periphery) and the independent or predictor variables (the eight risk factors for diabetes). The regression analysis was designed to account for the possibility of

interactions between the risk factors, providing a valid means of identifying the most relevant risk factors in the pre-specified list. This model identified which risk factors were significant predictors of early colour vision loss and rod and cone sensitivity loss. Figs. 20 and 21 show the results of the multiple regression analysis, with CAD and rod and cone sensitivity as dependent variables.

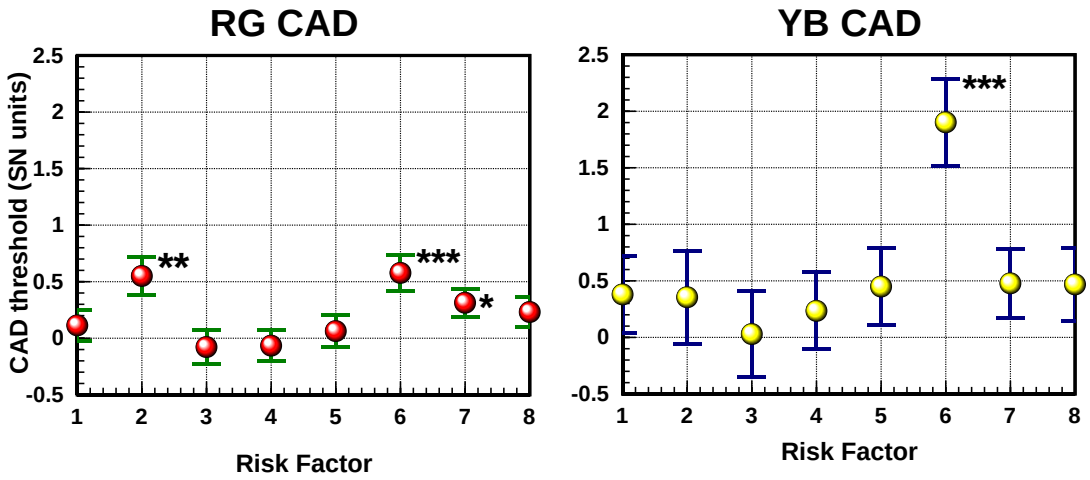

Figure 19: Output of the model showing the relationship between RG CAD and YB CAD as dependent variables, with smoking (1), hypertension (2), family history of diabetes (3), unhealthy diet (4), fasting blood sugar (5), age (6), lack of exercise (7) and elevated B

Note: Statistical significance shown: *** $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$. (A) The significant predictors for elevated RG CAD threshold, starting with the highest, are age (beta = 0.29, $p < 0.0001$), hypertension (beta = 0.29, $p = 0.002$) and exercise (beta = 0.20, $p = 0.013$). (B) The only significant predictor for elevated YB CAD threshold is age (beta = 0.39, $p < 0.0001$).

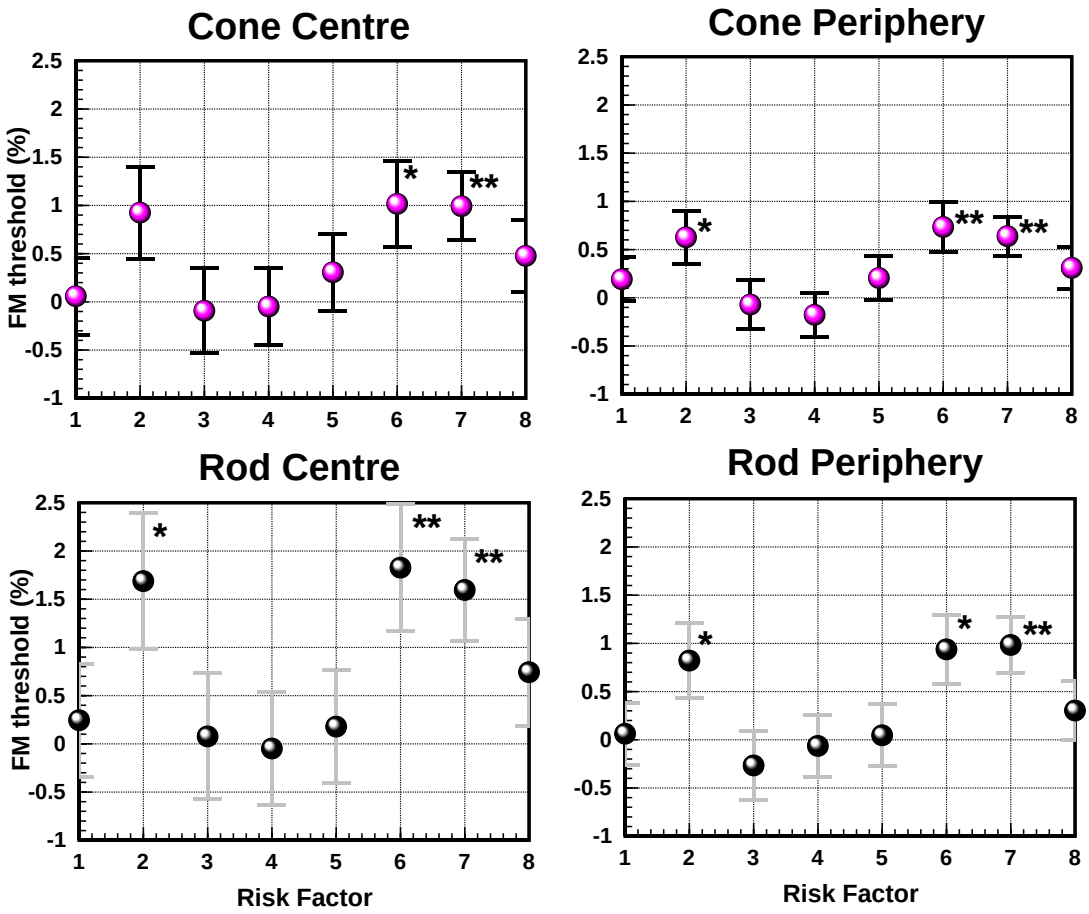

Figure 20: Output of the model showing the relationship between rod and cone sensitivity as dependent variables.

Note: The figure is divided into top and bottom. Top: the significant predictors for a decrease in cone sensitivity, starting with the most significant, are exercise (7) (p = 0.006 in the centre, p = 0.002 at the periphery); age (6) (p = 0.025 in the centre, p = 0.005 at the periphery); and hypertension (2) (p = 0.023, only at the periphery). Bottom: the significant predictors for a decrease in rod sensitivity, starting with the most significant, are exercise (7) (p= 0.003 in the centre, p = 0.001 at the periphery); age (6) (p = 0.007 in the centre, p = 0.001 at the periphery); and hypertension (2) (p = 0.018 in the centre, p = 0.034 in at the periphery).

There were significant differences in the high-risk group's measurements for RG and YB colour vision and rod and cone sensitivity, compared to the normal group. The results revealed that the main risk factor that affects both RG and YB loss is being

over 45. For rod and cone sensitivity, the main risk factors were lack of exercise, being over 45, and having hypertension. Surprisingly, the risk factors that did not cause a significant deterioration in visual performance in this study were smoking, family history, unhealthy diet and a fasting blood glucose level above 100mg/dl.

The outcome of the regression analysis led to the isolation of several risk factors that were important for the loss of colour vision in high-risk subjects, and which therefore may be predictive of the future development of diabetes and/or diabetic retinopathy or visual dysfunction. Although correlation strengths are noted in the results tables, caution is advisable when drawing conclusions about the most significant risk factors in predicting disease development or the prognosis of subjects, as a longitudinal study was not adopted. These issues are discussed in the following chapter, which analyses the implications of the risk factor assessment for future research and practice.

4.6 Results summary

The comparison of subjects with diabetes and those with a high risk of diabetes revealed notable similarities in the incidence of visual loss in these groups, particularly when it came to colour vision deficiency. The defects observed in the high-risk group tended to be more diffuse and variable than those seen in the diabetic group, although similar patterns of loss were noted. It was also observed that VA and FCS changes were not consistent across the high-risk and diabetic groups, suggesting some differentiation in the stages of loss of visual function over time or as part of the diabetes disease process. From this data, one can postulate that chromatic sensitivity changes are involved in the early stages of loss of visual function, while changes in VA and FCS may occur at later stages or following the onset of clinical diabetes.

The findings also confirmed that high-risk subjects experienced chromatic sensitivity changes that were not experienced in healthy control subjects. However, FCS changes were not any more evident in high-risk subjects at this stage compared to the normal subjects. VA changes showed an intermediate level of visual function loss, potentially reflecting a gradual loss of visual function over time. The comparison of high-risk subjects to normal subjects highlighted the significance of visual changes in the early stages of diabetes.

This section has provided an insight into the accumulation of specific risk factors for diabetes in the study's target group (those at a high risk of developing diabetes). The data set shows that risk factors were common among subjects with diabetes and those at a high risk of diabetes, which reflects the inclusion criteria applied to these groups in the study design. However, specific risk factors were more common in subjects with established diabetes; these were less frequently present or less pronounced in the high-risk group. This reflects the progressive nature of the diabetes disease process and the accumulation of risk factors over time. Certain risk factors present in the high-risk group were of specific importance because this group showed signs of early colour vision loss. Therefore, those risk factors may be associated with colour vision loss and predict further vision loss at a later stage in the development of diabetes.

Analysis of the risk factors in this subject group found that people over 45 years of age, having hypertension, and / or lack of exercise were all associated with a loss of colour vision. These findings were generally consistent across most of the colour vision parameters assessed in the study, although the only feature that was consistent across every parameter was age. These risk factors reflected both modifiable and non-

modifiable risk factors, which may be of specific relevance to the clinical approach to managing and preventing diabetes. Furthermore, the lack of significance of other risk factors associated with diabetes in the context of colour vision loss provides a refined focus for future research and practice.

Overall, these results demonstrate that subjects with a high risk of diabetes form a distinct group in terms of their visual functioning. They demonstrate differences in colour vision, rod and cone sensitivity, VA and FCS. They also have specific risk factors. Colour vision loss can occur at an early stage in these subjects, acting as an intermediate marker that distinguishes between the healthy population and the population with diabetes.

5. Discussion

5.1 Overview

The previous chapter provided an overview of the results of the study. Detailed comparisons were made between the indices of visual performance measured in normal subjects, in subjects at high risk of developing diabetes and in those with clinical diagnosis of diabetes. These findings are discussed critically in this chapter and related to what is already known, particularly in relation to colour vision changes in diabetes.

5.2 Colour vision loss in pre-diabetic subjects

One of the aims of this investigation was to evaluate the degree to which subjects with diabetes exhibited changes in colour vision consistent with previous findings in the literature. Previously published studies have shown a correlation between diabetic retinopathy and loss of chromatic sensitivity (Fong et al., 1999; Ong et al., 2003; Shin et al., 2005; Shin et al., 2014). Another study by Feitosa-Santana et al. (2010) also identified this loss in subjects with diabetes who showed no evidence of diabetic retinopathy. Although these studies employed different inclusion criteria and different methods of assessing colour vision, the consistency in their overall findings supports the link between diabetes and colour vision loss. A study by Barbur et al. (2012) found that 70% of 219 well-controlled diabetics in Abu Dhabi (who had no signs of retinopathy) showed significant losses of both RG and YB chromatic sensitivity. This study also revealed that there was no correlation between loss of chromatic sensitivity and the risk factors normally linked with diabetes such as daily glucose level, BMI, duration of diabetes and HbA1c. This may suggest that the neural changes in the

retina that cause significant reductions in chromatic sensitivity are not necessarily linked directly to insulin deficiency or an inability to use insulin effectively. In other words, a genetic predisposition for developing diabetes may be accompanied by other changes that affect the neural retina quite independently of diabetes, causing a loss of chromatic sensitivity. The only correlation with the loss of chromatic sensitivity in this study were the facts that all the subjects had been clinically diagnosed and were undergoing treatment for diabetes.

It was not clear from these findings when the loss of chromatic sensitivity occurred in the diabetic disease process. This is an important factor if considering colour vision as a screening tool for diabetic eye disease, as targeting the screening programme at an appropriate stage in the pathological process would be crucial in maximising the value of the screening programme (Dubrow et al., 2018). In response, this study was designed to investigate whether non-diabetic subjects at risk of developing diabetes already showed losses of RG and/or YB chromatic sensitivity that were significantly different to normal subjects. If this turned out to be the case, then the assessment of RG and YB chromatic sensitivity could, indirectly, become an important factor in the detection and management of diabetes.

In this study, 213 subjects were assessed. It was found that colour vison was affected in the group of subjects who were at a high risk of developing diabetes. The results revealed that over half of the high-risk subjects had thresholds above the median value for normal trichromatic vision, with 32% and 41% of subjects above the +2.5σ upper limit for the RG and YB thresholds, respectively (see Fig. 12 and Fig.13). That said, although the high-risk subjects exceeded the median values for normal subjects,

the majority of data remained within the upper limits of normal variability. In the diabetes group, however, no subject had RG or YB thresholds below the median, and 39% and 83%, respectively, had RG and YB thresholds above the +2.5σ upper limit.

As previously mentioned, Feitosa-Santana et al. (2010) explored the changes in colour vision in subjects with diabetes without evidence of diabetic retinopathy. In other words, they examined subjects who were in the earlier stages of the disease or who experienced the disease without any apparent ocular neurodegenerative changes. This study employed the Cambridge Colour Test (CCT) and the Lanthony desaturated D-15d test. The CCT showed diffuse loss of colour vision while the D-15d test revealed only tritan losses. The authors recognised the advantages of the CCT over the D-15d test but noted the need for further studies to confirm and verify these findings. In this study, all of the participants (31 diabetic subjects without signs of diabetic retinopathy and a control group of 61 subjects) were aged over 50, which may have introduced age-related effects on the colour vision losses that were observed. CCT does not have a normal standard observer but the normal ranges of the CCT thresholds were determined as the 95% boundary for the controls. Therefore, the present study, which employed the CAD test and focused on a younger population, had some methodological advantages over the approach used by Feitosa-Santana et al. (2010). In this study, the CAD's computation of RG and YB colour thresholds related almost linearly to the corresponding cone contrasts generated by the coloured stimulus, suggesting that the magnitude of the measured thresholds provided a good indicator of the severity of the colour vision loss. The details of the CAD test can be found in Rodriguez-Carmona et al., (2012). In the CAD test, both the RG and YB thresholds are based on a standard normal observer which takes age into account. The

CAD test has been shown to be very accurate in detecting the mildest colour defects and classifying the type of colour deficiency based on whether it is congenital (protan, deutan or tritan) and/or acquired (Rodriguez-Carmona et al., 2021).

In the study by Feitosa-Santana et al. (2010), the level of colour vision loss ranged according to the test employed. This is not unexpected. With the D-15d test, 6.5% of subjects were found to have severe colour vision loss, whereas the CCT showed that 19% of subjects had severe colour vision loss. This is likely due to differences in the sensitivity of the tests, with the CCT revealing higher sensitivity. However, there was no gold standard against which to compare these two testing protocols in the study.

In the present study, it was specifically separated the subjects into diabetic and high-risk groups based on inclusion criteria, then the CAD test was used to account for age, leading to more sensitive measurements. The visual functioning of the diabetic subjects was negatively affected to a greater extent than it was in the high-risk and normal groups. The high-risk subject group also had increased RG and YB thresholds and worse rod and cone sensitivity compared to the normal group. These findings suggest that colour vision and rod and cone sensitivity may be affected early in the diabetes disease process.

5.3 Potential mechanisms involved in diabetes

This study reveals clear loss of colour and rapid flicker sensitivity in people classed as being at risk of developing diabetes, well before they can be clinically diagnosed with diabetes. Spatial vision is less sensitive as a result of larger variability caused by potential, accompanying changes in the optics of the eye. As a result, VA and FCS

are less sensitive and more likely to reveal significant changes in diabetic patients. This section considers the potential mechanisms that might account for this early loss in visual function as revealed in colour and rapid flicker sensitivity tests.

There have been multiple attempts in the literature to explain the deterioration in colour vision observed in diabetes. A study by Gella et al. (2015) suggested possible mechanisms for these findings with an origin in the different stages of the visual processing pathway, i.e. pre-receptor filtering (the lens, macular pigment), cone photoreceptors, and post-receptor processes (RG channel, S-cone and luminance channels). The pre-receptor eye structures, including the lens, are particularly susceptible to the pathological processes linked to diabetes. In subjects with diabetic retinopathy, researchers have reported an association between colour vision loss and cataract susceptibility (Kessel et al., 2010). It is also well known that yellow chromophores accumulate inside the lens with increasing age, reducing the transmission of blue light, leading to YB defects (Gella et al., 2015). In diabetes, this process is accelerated due to enzymatic glycation of the lens proteins and browning of the lens (Kessel et al., 2010). This has been backed up by a study by Ao et al. (2019), which found that age-related cataracts make the loss of colour perception more pronounced, including the spectra associated with RG and YB loss in diabetes. Due to the younger age of the high-risk group in our study and the exclusion criteria adopted, neither cataracts nor lens yellowing is likely to explain the results.
Factors that affect the integrity or function of photoreceptors within the retina may also affect chromatic sensitivity in diabetics. For instance, Gella et al, (2015) demonstrated that many subjects with diabetes experience increased intraocular pressure (IOP). The mechanism accounting for the loss of colour vision linked to

raised IOP relates to short-wavelength cones being less likely to resist pressure increases, with YB ganglia morphology and connectivity more susceptible to IOP-related damage (Jacobs, 2013). However, changes in IOP typically develop several years after the diabetes process has begun (Briggs et al., 2016). Therefore, it is unlikely that this accounted for the loss of colour vision observed in high-risk subjects in this study.

While an increased risk of cataracts or raised IOP may be associated with diabetes, it also important to consider the microvascular effects in diabetes and the impact on neuronal function. Microvascular dysfunction has been recognised in subjects with diabetes and pre-diabetes (Sorensen et al., 2016), and it has been proposed that it may be linked to the neuropathic changes seen in subjects with pre-diabetes (Papanas et al., 2011). As acquired colour loss is often associated with neurodegenerative symptoms (Gella et al., 2015), it is therefore plausible that microvascular and/or neuropathic changes precede diabetic retinopathy, thereby contributing to chromatic sensitivity loss.

It has also been reported in the literature that retinal sensitivity is decreased in diabetic subjects, and the photoreceptor layer of the retina is less thick (measured with OCT), even when microvascular changes are not evident (Verma et al., 2009). However, the study by Gella et al. (2015) failed to support this observation in subjects with diabetes who lacked diabetic retinopathy, regardless of whether they experienced changes in colour vision. In other words, the current literature fails to provide clear evidence of the association between the thickness of the photoreceptor

layer and chromatic sensitivity in diabetes. Studies of this association in pre-diabetics or subjects at a high risk of diabetes have not yet been performed (Wolff et al., 2015).

Some studies have suggested that early subclinical vascular changes may be evident in the eyes of subjects with diabetes who do not have diabetic retinopathy. These changes may contribute to colour vision loss and broader vision loss. Talisa et al. (2015) used OCT angiography to evaluate the eyes of subjects without diabetic retinopathy. They noted foveal microvascular changes that would normally be missed in routine eye screening. Garcia-Martin et al. (2019) have recently suggested that subclinical ischaemia may contribute to colour vision loss in the early stages of diabetes. They examined subjects with early diabetes and good metabolic control without evidence of retinopathy and found that they showed visual dysfunction consistent with retinal neurodegeneration. Subclinical ischaemia may account for neuronal damage before the emergence of other vascular changes in the retina, although Garcia-Martin et al. suggested further studies are needed to explore this possibility. However, Sohn et al. (2016) found that people with diabetes with no or minimal diabetic retinopathy exhibited retinal diabetic neuropathy but showed no clear evidence of microvascular damage. The same study found that retinal neuropathy was degenerative and progressive in nature but not ischaemic in origin.

Rod and cone photoreceptor defects have been noted in patients with diabetes and/or diabetic retinopathy to varying degrees in the literature (Roy et al., 2017). For instance, Holopigian et al. (1997) found that cone changes in a-waves were notable in the sensitivity parameter, implying transduction abnormalities in cones that could lead to defects in colour vision. A-waves are a component of an electroretinogram

(ERG). An ERG is a diagnostic test that is employed to measure the electrical activity generated by neural and non-neuronal cells in the retina in response to a light stimulus. A-waves refer to initial corneal-negative deflection. These waves are derived from the cones and rods located in the outer photoreceptor layers. In this study, the rods were similarly affected, but the authors did not explore the impact of these findings on colour vision (Holopigian et al., 1997).

A study by Tyrberg et al. (2011) demonstrated that patients with recent-onset diabetes show deficits in both rod and cone signalling, even in the absence of visible retinopathy. In this study, both a-wave and flicker reaction times were delayed in patients with diabetes compared with control subjects. A study by Elsner et al. (1988) also suggested that abnormal photo-pigment bleaching is a characteristic of diabetes because diabetic subjects in this study required more light than control subjects to bleach an equivalent amount of photo-pigment. Nevertheless, further studies are needed to explore these mechanisms and to identify precisely how microvascular and neuropathic mechanisms contribute to vision loss in subjects with diabetes, which may make it possible to target the screening process at an appropriate stage in eye disease development.

5.4 Diabetes progression over time

It has been shown that colour vision defects may change over time in diabetes, predominantly in the form of YB loss (Barbur et al., 2012). The results of the current study confirm this, showing a more diffuse (non-axis-specific) loss in subjects at a high risk of developing diabetes and a more pronounced YB loss (in comparison to RG loss) in subjects with a diagnosis of diabetes. The results also suggest a

progressive loss of colour vision when comparing the high-risk and diabetic groups. However, Barbur et al.'s (2012) study suggested that there was no correlation with diabetes duration (see Section 2.4.4), so more work might be needed to establish a link between the level of chromatic sensitivity loss and the severity of the diabetes disease process.

As well as the deficits in colour vision that were observed in the present study in high-risk and diabetic subjects (compared to the healthy population), several other visual deficits were also observed in these two groups. Both VA and FCS were reduced in subjects with diabetes compared to the healthy population (as shown in Tables 11 and 13). In subjects with a high risk of diabetes, FCS (particularly under mesopic conditions) was also reduced compared to the healthy population (see Table 13). The findings of the rod and cone sensitivity test were also surprising, revealing that both rod and cone sensitivity were adversely affected in high-risk and diabetic subjects. Interestingly, this study suggests a greater loss in rod and cone sensitivity in the central region compared to the periphery (see Section 4.4.2). These findings generally support the idea that a broad range of visual characteristics are adversely affected by the diabetes disease process and are also evident in the early stages of that process. This finding, however, contradicts that of previous studies, such as Barbur et al. (2012), which suggested that VA is practically unaffected, and flicker sensitivity only marginally affected, in comparison to chromatic sensitivity loss.

Studies by Sokolowska-Oracz and Piatkiewicz (2017) and Vadivel and Vijayamalathi (2016) have shown an association between colour vision abnormalities in subjects with pre-diabetes (see Section 2.4). However, these two studies used conventional

VA and FM 100 Hue arrangement tests to examine their participants. As a result, the assessment of VA and colour vision may well have been less specific and less sensitive. It might have been less capable of detecting visual impairment than the computerised and quantitative psychophysical tests used in our study (the Acuity-Plus and CAD tests).

A recent study of impaired colour vision by Piro et al. (2019) suggested that diabetes might lead to disruption of the retina-brain cortex pathways. The role of optic nerve involvement in more advanced stages of the disease, as well as the increasing incidence of ischaemic changes in subjects with diabetes, may account for the deterioration in colour vision observed in high-risk and diabetic subjects. Therefore, the mechanism used to account for this must consider this pattern. More studies are needed to not only establish a correlation between events but also to longitudinally analyse changes in colour vision and identify possible mechanisms. These studies should also take into account other changes in vision (such as acuity) in subjects with early-stage diabetes or who are at a high risk of diabetes.

The evidence suggests that subjects with diabetes experience a range of visual defects and loss of vision over time, reflective of several cardiovascular and diabetes-specific mechanisms, as well as the effects of age (Barbur et al., 2012; Barbur et al., 2015). This study found that VA was one key visual measure that was adversely affected in both groups (subjects with diabetes and subjects at a high risk of diabetes) in comparison with the normal group. Studies have shown that diabetic retinopathy and diabetic macular oedema may be associated with a loss of VA (Maheshwary et al.,

2010). Also, previous data have suggested that VA may be significantly affected in subjects with pre-diabetes (Sokolowska-Oracz and Piatkiewicz, 2017).

It may be that the differences in these results are due to the testing methods used (a standard Snellen chart as opposed to the Acuity Plus test). Nevertheless, there is some disagreement over the loss of VA observed in pre-diabetic and diabetic subjects. This disagreement may also stem from the heterogeneity of this subject population, particularly in terms of the diabetic risk factors present and the sociodemographic make-up of the subjects. What is clear, though, is that there is an established pattern of deterioration in VA and other visual features in subjects with diabetes over time as neuronal and vascular damage accumulate secondary to the disease process (Qiao et al., 2015). Therefore, it is reasonable to assume that similar mechanisms that result in loss of colour vision and visual performance may be present in this population and apparent in subjects with a high risk of developing diabetes.

5.5 Identification and grading of risk factors for diabetes

A key aim of this study was to determine which of the diabetic risk factors identified by the ADA (2003, see Table 2) have the biggest effect on diabetes, based on a visual assessment. It was thought that these might be able to predict the onset of diabetes in the future.

Eight of the most important, modifiable risk factors for diabetes were analysed using multiple regression analysis. The following factors had the largest impact on visual functioning in subjects at a high risk of diabetes: age over 45 years, hypertension and a lack of exercise. Only advanced age was associated with increased YB loss in this

subject group, but all three risk factors were significantly associated with other losses in visual function, including RG sensitivity and rod and cone flicker sensitivity (at both the centre and the periphery). These findings are promising and suggest that different risk factors associated with diabetes have varying levels of prominence in the development of diabetes.

Age over 45 years was a key risk factor identified in this study. Being over 45 is notably associated with an accumulation of disease processes and additional risk factors that can contribute to specific pathological processes in diabetic subjects, such as microvascular disease and cataracts (Zoungas et al., 2014). Advanced age among subjects at a high risk of diabetes may also be associated with a long period of subclinical hyperglycaemia or retinopathic changes. Therefore, it is not surprising that advanced age is linked to a deterioration in colour vision in high-risk subjects, although the exact reasons underlying this link remain unknown.

Hypertension is another key risk factor associated with diabetes. It was specifically found to have a negative impact on chromatic sensitivity in this study. Hypertension is associated with a risk of cardiovascular disease and is a common factor involved in ocular disease (Bhargava et al., 2012). Hypertension is also linked to dietary factors and physical activity, hence complicating its identification as an independent cause of colour vision loss (Bhargava et al., 2012). Similarly, the final risk factor of note in the present study was a lack of exercise, which is widely acknowledged as a contributing factor to obesity and the poor lifestyle habits that can increase the risk of diabetes (Chudyk and Petrella, 2011). Both hypertension and lack of exercise are indicative of

lifestyle choices that are obesogenic, placing individuals at an increased risk of vascular and cardiovascular disease.

Overall, the grading of these risk factors may serve as an important tool to predict the onset of diabetes, although longitudinal data would be needed to validate this. However, the potential to evaluate the risk of diabetes through early signs of colour vision loss and the presence of key risk factors in subjects who have not yet been clinically diagnosed with diabetes is an important outcome of this study. Risk factors and early colour vision screening could be combined to provide a basis for predicting diabetes and making early interventions, including the use of preventative measures to minimise the progression of the disease (Siu, 2015). Larger studies would be needed to confirm these findings, and it is also worth considering the cost of colour vision tests as a routine part of diabetic screening programmes (Siu, 2015).

5.6 Limitations

There were several limitations to this study. This section details the steps taken to minimise their effects. It should first be noted that this study was not intended to provide a basis for evaluating the accuracy of visual assessment tests as a screening tool for diabetic eye disease. Rather, the study was intended to confirm the findings of previous studies done in diabetic patients and to add to the data set regarding the timing of colour vision loss subjects at a high risk of developing diabetes, while also providing additional insights into other visual parameters and deficits in these subjects. Therefore, while this study suggests that colour vision testing may be a potential screening tool for diabetic eye disease in the future, the results do not

provide a basis for evaluating the feasibility, sensitivity, or specificity of this testing protocol for application in screening programmes.

One of the most obvious constraints of the study was the relatively short amount of time that was available to carry out all of the tests for each subject. This meant that subjects were examined binocularly, even though monocular testing would have been more effective at measuring structural changes in the retina and correlating them with functional losses in vision. However, this was not the aim of the current study. Moreover, since pre-diabetics subjects were the main target, it was not expected that they would have significant structural changes since they had not been clinically diagnosed with diabetes. Testing each eye separately would have increased testing time and may have led to more varied results. Since it was not necessary to identify correlations with structural changes, the only disadvantage of using binocular assessment was that the thresholds probably reflected the performance in the eye that was least affected; monocular tests would probably have shown even greater changes in the eye that was most affected. The pros and cons of monocular and binocular testing were considered carefully at the start of the study, and binocular testing was eventually chosen. The previous study on diabetics by Barbur et al. (2012) was also carried out binocularly for the same reasons, and the study revealed considerable losses of chromatic sensitivity; these would have been even greater if only the most affected eye had been examined.

The project gathered cross-sectional data collected at a single time point, which was used to explore differences in the association between variables in different subject groups. As mentioned previously, this approach was preferred to a longitudinal study,

which was not considered feasible or necessary in this instance. Evaluating changes in colour vision in diabetic or high-risk subjects over time would have required a lengthy follow-up period. This would not have been practical for the present study and would not have provided any additional insight into the study's specific research aims and objectives.

Another limitation of the present study was the relatively high dropout rate of participants who were initially enrolled. There was a general reluctance of subjects to participate in the study at all, and those who were approached were unwilling to undergo optical testing. In an attempt to minimise the dropout rate, more information about the tests was communicated to the potential participants before they enrolled. For example, the participants were informed of the time needed to complete all the tests (80-90 minutes). For future studies, it is recommended that researchers provide participants with a careful explanation of the type of tests and the purpose of the study before taking part; understanding the testing process is key to ensuring compliance and acceptability, thereby maximising participation.

A further limitation was the fact that it was not possible to conduct OCT imaging for subjects with pre-diabetes or high-risk subjects at the Al Shalahi Specialized Centre, as this was not part of their protocol. In an attempt to limit any confounding factors that could influence the results, any subjects who showed abnormal VA or presented with an underlying health issue were excluded from the data analysis. These issues were revealed by the questionnaire (see Appendix A). The use of OCT would have provided an opportunity to explore further anatomical features of the retina that may

have revealed the mechanism behind the colour vision loss. This should be considered in future studies.

Finally, it should be noted that the blood glucose data used to identify subjects at a high risk of diabetes was self-reported. As such, the lack of HbA1c results for this population is another limitation. Therefore, future studies should consider implementing HbA1c blood tests for high-risk subjects to ensure that an accurate comparison can be made with the subjects with diabetes.

6. Conclusions

6.1 Overview

This final chapter reflects on the overall results of the study and the relationship between the reported findings and the initial aims and objectives. This will include an assessment of the methods used, their limitations and their implications for the analysis and interpretation of the data. The chapter will provide some broad insight into the significance of these findings within the context of contemporary practice. Finally, some suggestions are made about how these findings might influence future research and practice.

6.2 Key findings and significance

As diabetes continues to become more prevalent, it is necessary to establish new and improved methods of diagnosis. To this end, the current study has focused on determining the role sensitive, functional vision tests, such as those employed in this study, can play in assessing colour and rapid flicker sensitivity changes in those of increased risk of developing diabetes. The study concentrated mostly on examining subjects who presented with at least three of the accepted risk factors for diabetes, as identified by the ADA. Equally important, the study examined the subjects in this high-risk group to determine whether the measured changes could be attributed to any particular shared risk factors or genetic predispositions.

This study has confirmed that subjects with diabetes, but without any clinical signs of diabetic retinopathy may still experience a loss of chromatic sensitivity. 23 subjects with clinically diagnosed diabetes demonstrated significant losses of colour vision.

This was in accordance with previous studies, and the findings provide conclusive evidence that diabetic disease or some other factor closely linked to diabetes can cause significant loss of RG and YB colour vision. The findings show that while such losses predominantly affect the YB colour mechanisms, there is also significant loss in the RG chromatic sensitivity in almost every subject with YB loss. In comparison with the diabetic group, the high-risk group tended to show smaller and more balanced loss of both RG and YB chromatic sensitivity. This highlights the more widespread effects of established diabetes on visual function, as well as the particular importance of early changes in colour vision loss, which can precede losses in VA or FCS in many subjects.

A novel finding from this study involves the loss of sensitivity in other attributes of vision such as spatial vision and rapid flicker. Specifically, the study revealed that there were significant statistical differences in the VA and FCS of high-risk subjects compared with diabetic subjects. Statistically significant differences in VA were found for all of the parameters, including both positive and negative contrast under photopic and scotopic conditions. While the results suggest that VA and FCS (measured using the Acuity-Plus protocol) may be adversely affected as an early event in the diabetes disease process, mesopic FCS was generally similar between normal subjects and the high-risk group. Such findings are not surprising since it is well known that FCS is a more sensitive measure of spatial vision when compared to VA (Elliott & Flanagan, 2007). In addition, any form of spatial vision test relies on the quality of the retinal image in addition to the normal functioning of the retina and the visual pathways. The involvement of a larger number of variables that can affect the outcome of VA or FCS tests results in increased variability and reduced

sensitivity. The four parameters measured with the Acuity-*Plus* test provide a good assessment of the subject's spatial vision, but the increased inter-subject variability as a result of the many parameters that can affect the outcome of the test means that small changes are no longer statistically significant. The results from this study reveal significant differences in spatial parameters when comparing normals and diabetics, simply because the latter show greater loss of spatial vision, but not between normal and subjects at risk of developing diabetes, particularly in the mesopic range when larger pupil sizes and greater variability in higher order aberrations are involved. One of the advantages of CAD and Flicker-*Plus* tests is that neither is strongly affected by the quality of the retinal image. This is simply because the tests employ very large stimuli and the task does not require detection of fine spatial details, when the quality of the retinal image is important. These findings suggest that the two most sensitive visual parameters one can use to reveal changes in retinal sensitivity linked to diabetes involve the measurement of chromatic and rapid flicker sensitivity.

The findings from this study help us provide adequate answers to each of the objectives set up at the beginning of the project. The results show that:

1. The majority of subjects classified as being at risk of developing diabetes show significant losses of RG and YB chromatic sensitivity that can be measured reliably using the CAD tests.
2. Subjects at risk of developing diabetes also show a loss of rapid flicker sensitivity and those classified as diabetics also show a significant loss of spatial vision.
3. Not unexpectedly, there is a progression in the loss of visual sensitivity when comparing normal trichromats with subjects classed as being at high risk of developing diabetes and with subjects clinically diagnosed with diabetes. In

addition to significant loss of rapid flicker and chromatic sensitivities demonstrated in subjects at risk of developing diabetes, the worsening of vision in the diabetics group causes larger differences in spatial vision which become statistically significant, particularly in the mesopic range. The magnitude of the losses involved in some subjects is surprising, given the relatively normal VA results measured using clinical tests.

The results of the rod and cone sensitivity test were also surprising in that they suggested differences in rod and cone sensitivity between the diabetic and the high-risk groups. The differences between these two groups were statistically significant for all of the eccentricities. Also, the results revealed reduced rod and cone sensitivity in subjects with a high risk of diabetes when compared to normal subjects. This finding was largely uniform in nature (affecting all of the regions analysed), but it seems to have been slightly more pronounced in the central region than the peripheral region. The results from the diabetic group also showed that rod and cone sensitivity was particularly affected in the central foveal location, this could be due to the duration of diabetes and exposure to retinal hypoxia, both of which are important elements of the diabetic disease process along with photoreceptor degeneration. Hypoxic conditions caused by vasculopathic chages in the retina and other insulin related, neuropathic processes can lead to diminished functioning and cellular apoptosis with the ultimate loss of ganglion cells.

This study also explored the relationship between the level of chromatic sensitivity loss and the known risk factors for diabetes. Of the eight commonly identified risk factors, three had higher correlations with colour vision loss when assessed using

multiple regression analysis. The three most prominent risk factors were being over 45 years, doing little or no exercise, and suffering from hypertension. Although these findings may be of immediate clinical interest, it remains to be established how these factors correlate with the neural changes that are directly linked to the deterioration of colour vision and rod- and cone-mediated flicker sensitivity.

A range of factors associated with visual functioning in healthy normal subjects, patients diagnosed with diabetes and at risk of developing diabetes have been examined in this study. These groups were compared with a group of subjects identified as being at risk of developing diabetes. The results observed suggest that chromatic sensitivity loss is an early event in the diabetes disease process that may also pre-date other pathological changes related to diabetes, particularly those seen in the eyes.

6.3 Implications for future research and practice

Despite the findings of this study, several outstanding issues and controversies remain to be resolved. Firstly, as mentioned, an important limitation of the current study is its cross-sectional design. A potential future enhancement would be to design a longitudinal study that could examine subjects over time. A project for which more time was available could use the psychophysical measures reported in this study in conjunction with high-resolution imaging techniques to explore whether there is a relationship between structural changes in the retina (including photoreceptor damage) and vision function.

At a more practical level, identifying diabetes at an earlier stage, allowing for earlier interventions, might lead to cheaper and more effective treatments. Clinical control trials and a revision of the current screening programmes would both be necessary to evaluate the feasibility of earlier treatment options for diabetes. A screening programme that assessed chromatic sensitivity is the most promising option, particularly if colour vision screening could be carried out relatively quickly (i.e., in approximately two minutes) and was accurate and easily accessible. This would require a screening test with high levels of sensitivity and specificity, enabling the detection of abnormal YB and/or RG colour vision. This study has shown how chromatic sensitivity is affected in subjects who are not clinically diagnosed as diabetic. The impact of other retinal conditions on the early loss of chromatic sensitivity still needs to be examined.

From a geographical perspective, the high prevalence of diabetes in the Gulf countries means that this study is particularly pertinent to research and practice in that region. The prevalence of diabetes in Kuwait is increasing. This study, therefore, has the potential to make a timely impact on the methods used to detect and monitor the progression of the disease.

It is important to note, however, that the findings of the present study are unlikely to have any direct therapeutic implications for subjects with diabetes or who are at a high risk of the disease. Indeed, the study has found that while the levels of colour vision loss are significant for both these groups, the degree of loss would not be detectable via the routine vision assessments that they generally undergo. However, the results of this study do bear some significance when it comes to the strategies

used to stratify risk and identify the early visual changes that are associated with diabetes. Future studies could investigate potential early interventions to help with the management and prevention of diabetes, which could prove cheaper and more effective than current approaches.

6.4 Concluding remarks

The data obtained in this study have revealed significant differences between a healthy group of 40 subjects and a group of 150 subjects who were identified as being at a high risk of developing diabetes. The largest differences were observed in the measures for chromatic sensitivity (for both YB and RG) and rod and cone sensitivity. Differences were also identified for VA and FCS. As predicted, the diabetic group, composed of 23 subjects, had the worst thresholds in all of the visual performance measures that were analysed in this study.

The results suggest that the loss of chromatic sensitivity and the worsening of visual performance in other visual tasks are important, early events that can be used to detect the presence and to characterise the diabetic disease process. These vision changes can provide important insights into the changes taking place in the retina as a result of diabetes, even during the earliest stages when diabetes cannot be clinically diagnosed. The results of this study contribute to a better understanding of the loss of visual function in the early stages of diabetes and may help to design new experiments to elucidate both new and older questions and to link the measured changes in functional vision to specific mechanisms and structural changes in the retina that cannot be imaged easily using current imaging methods.

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Appendix A. Study questionnaire

Diabetes Risk Assessment Questionnaire

The aim of the following questionnaire is to assess dietary habits of people with, or at risk of, type 2 diabetes.

This questionnaire forms part of a PhD study to establish how and whether the loss of colour vision in those at high risk of developing diabetes affects a significant number of people. If this were the case, accurate assessment of RED/GREEN and YELLOW/BLUE thresholds would be an important risk factor in the pre-screening for type II diabetes. No personally identifiable data will be collected, stored or published.

You are under no obligation to complete this questionnaire. You do not need to decide today – feel free to take the questionnaire home with you, and drop it off at your next appointment if you decide to take part and leave your questionnaire in the box at reception.

If you have any questions about this research, please contact the lead investigator (details below):

Dr Qais Bastaki, PhD student, City, University of London

E-mail: [redacted]

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PART 1:

DIABETES RISK ASSESSMENT:
(Please tick the correct option)

1. Age

☐ Under 45 years ☐ 45–54 years
☐ 55–64 years ☐ Over 64 years

2. Body-mass Index

☐ Lower than 25 ☐ 25–30
☐ Higher than 30

3. How often do you usually do at least 30 minutes of physical activity, whether at work and/or for leisure (including normal daily activity)?

☐ Rarely ☐ Several times a week
☐ Less than once a week ☐ Nearly every day or daily
☐ Once a week ☐ Twice or more per day

4. How often do you eat vegetables, fruit or berries?

☐ Every day ☐ Not every day

5. Have you ever taken medication for high blood pressure on a regular basis?

☐ Yes ☐ No

6. Have you ever been found to have high glucose (e.g. in a health examination, during an illness, during pregnancy)?

☐ Yes ☐ No

7. **Have any of the members of your immediate family or other relatives been diagnosed with diabetes (type 1 or type 2)?**

☐ No

☐ Yes: grandparent, aunt, uncle or first cousin (but not own parent, sister, brother or own child)

☐ Yes: parent, sister, brother or own child

8. **Do you have a colour vision deficiency?**

☐ Yes ☐ No ☐ I Don't know

PART 2

HAVE YOU BEEN TOLD THAT YOU HAVE DIABETES?

(Answer as appropriate)

Yes – since when?.....

No – skip this part (all questions)

1. **Do other members of your family have diabetes?**

☐ Yes ☐ No

2. **Have you changed your diet since being diagnosed with diabetes?**

☐ Yes ☐ No

3. **Which medications are you taking, if any?**

.....

4. **Have you changed your lifestyle since being diagnosed with diabetes?**

☐ Yes ☐ No

5. **Have you made any changes <u>to your diet</u> since being diagnosed with diabetes, based on this advice?**

☐ No (please go to question 6)

☐ Yes (please describe the changes you have made)

.....

.....

6. **If you did not make changes to your diet, what was/were the reason(s)? (please tick all that apply)**

- I didn't receive any advice to change my diet ☐
- I didn't understand how it would help my eyes ☐
- Too expensive ☐
- My diet is already good ☐
- I/my family/partner do not like the foods recommended ☐
- Too much else to worry about / too much hassle ☐
- Other reasons (please specify) ☐

……

7. **Do you smoke cigarettes?**

- Yes (go to question 8) ☐
- Not now, but I did in the past (go to question 11) ☐
- No, never (go to question 12) ☐

8. **If you are a current smoker, please could you answer the following:**

How many cigarettes do you smoke, on average, per day?

……

For how many years have you been smoking?

……

9. If you are a current smoker, did your hospital eye care practitioner advise you to stop smoking when you were diagnosed with diabetes?

Yes ☐

No ☐

Can't remember ☐

10. If you are a current smoker, and your hospital eyecare practitioner did advise you to stop smoking, what were your reasons for not stopping?

I enjoy smoking ☐

I don't see how it will help my eyes ☐

It's too difficult to stop ☐

Other (please specify) ☐

……..

……..

11. If you used to smoke cigarettes but have given up, please could you answer the following:

How many cigarettes did you used to smoke, on average, per day?

……..

For how many years did you smoke?

……

Did you stop smoking because of your eye condition? Yes ☐

No ☐

12. Other than verbal advice and information, were you provided with any of the following by your hospital eye care practitioner? (please tick all that apply)

- An information sheet / leaflet about lifestyle changes (e.g. diet and nutritional supplements, stopping smoking) ☐
- Information about support groups, such as the Macular Society ☐
- An information sheet / leaflet about your eye condition ☐

Many thanks for your help with this questionnaire. If you have any questions about this research, please contact Dr. Qais Bastaki.

E-mail: [redacted]

For administrative use	RE	D	W	N	LE	D	W	N

Appendix B. Participant information sheet

Chromatic sensitivity changes in subjects with high risk of developing diabetes

We would like to invite you to take part in a research study. Before you decide whether you would like to take part, it is important that you understand why the research is being done and what it would involve for you. Please take the time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

This study will investigate whether there is a loss of colour vision in subjects 'at risk' of developing diabetes and/or in subjects diagnosed with diabetes.

Why have I been invited?

We are carrying out this study on subjects who have a number of accepted risk factors for diabetes such as age, abnormal HbA1c, blood pressure, family and smoking history, or high Body Mass Index (BMI).

Do I have to take part?

No - it is up to you whether you decide to take part or not. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. You can withdraw at any time without giving a reason. You do not have to answer questions that you feel are too personal or intrusive, and to do so will not affect any future treatment. You can withdraw at any stage of the project without being penalised or disadvantaged in any way.

What will happen if I take part?

This research study involves collecting information from you via a questionnaire and by assessing your vision using non-invasive tests, such as the Colour Assessment and Diagnosis (CAD), Visual Acuity^{+} test and the Rod/Cone sensitivity tests.

For this study, you will attend your normal appointment at the Obeid Al Shelahi Specialized Center, and then we will ask you to perform 3 tests: the CAD test, 'Rods and cones' test and the Acuity^{+} test. This will take approximately 1 hour.

The research study will last for three years and we may ask you to do these tests one more time if necessary.

What do I have to do?

You will be asked to:

- Fill in a questionnaire providing some information about your medical history including some lifestyle markers, i.e., gender, age, diet, BMI.
- We will make sure your vision is corrected, if required.
- We will then measure certain parameters about your vision using the tests described earlier. The tests are easy to perform and require you to sit and look at a computer display. You will be given a response box. The instructions of each test will be explained to you at the start. You will see a target on the display and asked to respond in a particular way using the response box. You will also carry out a quick test to ensure you are familiar with the response box and the test.
- If you wish to stop the test at any point, for any reason, you will be able to do so.

What are the possible disadvantages and risks of taking part?

It is highly unlikely that the tests performed will cause any harm to you, since all measurements are non-intrusive. However, if any issues emerge during the course of the study, we will seek appropriate advice within the Center and take any necessary action to ensure your wellbeing is intact.

What are the possible benefits of taking part?

There are no direct benefits to you, however, longer term, the findings of this study may benefit health carers in the early diagnosis and treatment regimes of diabetes.

What will happen when the research study stops?

All information collected during the study will be processed and stored securely using password-protected systems. No personal or identifiable data will be stored. When the study is over, the data may be retained, but will be completely anonymous. All procedures are compliant with the Data Protection Act 1998.

Will my taking part in the study be kept confidential?

Only the members of the research team (mentioned below) will have access to the data. Your name will be replaced by a 'participant number' and your data will be saved under this code. Your name and personal details will be stored separately from the data in a locked filing cabinet. The files will be deleted using an appropriate program at the time.

Data will be kept for 10 years following City University guidelines on retention and then deleted using an appropriate program at the time.

What will happen to the results of the research study?

The study results will be analysed and presented at national and international meetings and in scientific journals. Identities of participating volunteers will not be revealed in any resulting published material. If you wish to be provided with a summary of the research findings at the end of the study, please place your initials in the appropriate box on the consent form.

PLEASE NOTE: Although these procedures may give you useful information about your vision, they are not a full eye test that can be used for diagnostic purposes and are no substitute for regular visits to your optometrist.

What will happen if I do not want to carry on with the study?

You are free to withdraw from the study at any time and without giving a reason. If you choose to do this, we will delete any existing information we have about you.

What if there is a problem?

If you have any problems, concerns or questions about this study, you should ask to speak to a member of the research team. If you remain unhappy and wish to complain formally, you can do this through Obeid Al Shelahi Specialized Center complaints procedure. To complain about the study, you need to phone. You can then ask to speak to Dr. Alduaij and inform him that the name of the project is: “Chromatic sensitivity changes in subjects with a high risk of developing diabetes”.

There will also be a complaint form available for you in case you would need one.

Who has reviewed the study?

This study has been reviewed and approved by the School of Health Sciences Research Ethics Committee at City, University of London, United Kingdom.

Thank you for taking the time to read this information sheet.

Appendix C. Consent form for study participants

Chromatic sensitivity changes in subjects with high risk of developing diabetes

Please initial box

1.	I confirm that I have had the project explained to me, and I have read the participant information sheet, which I may keep for my records. I understand this will involve: ● completing a diabetes risk assessment questionnaire ● performing non-invasive psychophysical tests to assess colour vision and night vision sensitivity ● allowing the researcher to have access to my medical records	
2.	This information will be held and processed for the following purpose(s): ● to perform required analysis for this study I understand that any information I provide is confidential, and that no information that could lead to the identification of any individual will be disclosed in any reports on the project, or to any other party. No identifiable personal data will be published. The identifiable data will not be shared with any other organisation.	
3.	I understand that my participation is voluntary, that I can choose not to participate in part or all of the project, and that I can withdraw at any stage of the project without being penalised or disadvantaged in any way.	

4.	I agree to City recording and processing this information about me. I understand that this information will be used only for the purpose(s) set out in this statement and my consent is conditional on City complying with its duties and obligations under the Data Protection Act 1998.	
5.	I would like to be provided with a summary of the research findings at the end of the study.	
6.	I agree to take part in the above study.	

_____ _____ _____

Name of Participant Signature Date

_____ _____ _____

Name of Researcher Signature Date

When completed, 1 copy for participant; 1 copy for researcher file.

استمارة الموافقة للدراسة بعنوان

التغيرات حساسية الالوان في المرضى الذين يعانون من ارتفاع مخاطر الإصابة بمرض السكري

يرجى المربع الأولي

1.	أؤكد أنني قد أوضحت المشروع، وقد قرأت ورقة المعلومات الخاصة بالمشاركين التي يمكنني الاحتفاظ بها لسجلاتي. وأنا أفهم أن هذا ينطوي على: • استكمال استبيان تقييم مخاطر السكري • إجراء الفحوص الفيزيولوجية الغير جراحية لتقييم رؤية اللون وحساسية الرؤية الليلية • السماح للباحث بالوصول إلى سجالتي الطبية	
2.	سيتم عقد هذه المعلومات ومعالجتها للغرض (الأغراض) التالية: أنني أفهم أن أي معلومات أقدمها سرية، وأنه لن يتم الكشف عن أي معلومات قد تؤدي إلى تحديد أي فرد في أي تقارير عن المشروع، أو إلى أي طرف آخر. لن يتم نشر أية بيانات شخصية قابلة للتحديد. لن يتم مشاركة البيانات القابلة للتحديد مع أي منظمة أخرى.	
3.	إنني أدرك أن مشاركتي تطوعية، وبإمكاني أن أختار عدم المشاركة في جزء أو كل المشروع، وأن أستطيع الانسحاب في أي مرحلة من مراحل المشروع دون التعرض للعقاب أو الحرمان بأي شكل من الأشكال.	

4.	أوافق على تسجيل المركز وتجهيز هذه المعلومات عني. وأنا أفهم أن هذه المعلومات سوف تستخدم فقط للغرض (الأغراض) المنصوص عليها في هذا البيان وموافقتي مشروطا المدينة الامتثال واجباتها والتزاماتها بموجب قانون حماية البيانات 1998.	
5.	أوافق على المشاركة في الدراسة أعلاه.	

_____ _____ _____

أسم المشارك تاريخ توقيع المشارك

_____ _____ _____

أسم الباحث تاريخ توقيع الباحث

عند الانتهاء، نسخة واحدة للمشارك. نسخة واحدة لملف الباحث.

Appendix D. Letters confirming ethical approval

Ethical approval from Kuwait Ministry of Health

State Of Kuwait
Ministry Of Health
Asst. Undersecretary for Planning & Quality

دولة الكويت
وزارة الصحة
وكيل الوزارة المساعد لشئون التخطيط والجودة

التاريخ: 28/5/2017
الرقم: 1356

To Whom it May Concern

***From**: Ministry of Health – Kuwait*

The Standing Committee for Coordination of Medical Research

*To : : **Qais Mohammed Bastaki***

Study title: <u>Chromatic sensitivity changes in patients with high risk of developing diabetes</u>
(#586/2017)

University of London City

The above mentioned Proposal was given an ethical approval by the Committee on its meeting held May 25, 2017

The research will be conducted in obeid Al shelahi specialized center

Asst. Undersecretary for
Planning & Quality

Head, Standing Committee for Coordination of Medical Research

Ministry of Health – State Of Kuwait

د/ محمد حاشم الرشيدي
وكيل الوزارة المساعد لشئون التخطيط والجودة

د/ محمد حاشم الرشيدي
وكيل وزارة الصحة بالإنابة

Hassan

Ethical approval from City University London

School of Health Sciences

Research Office
Northampton Square
London EC1V 0HB

Tel: +44 (0) 20 7040 5704

www.city.ac.uk

Ref: PhD/16-17/12

20 September 2017

Dear Qais, Marisa and John

Re: Chromatic sensitivity changes in diabetes

Thank you for forwarding amendments and clarifications regarding your project. These have now been reviewed and approved by the Chair of the School Research Ethics Committee.

Please find attached, details of the full indemnity cover for your study.

Under the School Research Governance guidelines you are requested to contact myself once the project has been completed, and may be asked to complete a brief progress report six months after registering the project with the School.

If you have any queries please do not hesitate to contact me as below.

Yours sincerely

[redacted]

[redacted]
Research Governance Officer

[redacted]

Appendix E.1: Normality test

Table E-1: Normality test using the Kolmogorov–Smirnov and Shapiro–Wilk tests. All the visual parameters in this study were non-normally distributed, so non-parametric tests were used.

	Kolmogorov–Smirnov			Shapiro–Wilk			Skewness	Test to be used
	Statistic	df	Sig.	Statistic	Df	Sig.		
phVA +	.255	213	.000	.490	213	.000	Positive	Non-parametric
phVA -	.285	213	.000	.426	213	.000	Positive	Non-parametric
phFCS +	.375	213	.000	.208	213	.000	Positive	Non-parametric
phFCS -	.279	213	.000	.491	213	.000	Positive	Non-parametric
meVA +	.243	213	.000	.582	213	.000	Positive	Non-parametric
meVA -	.234	213	.000	.587	213	.000	Positive	Non-parametric
meFCS+	.224	213	.000	.470	213	.000	Positive	Non-parametric
meFCS -	.129	213	.000	.898	213	.000	Positive	Non-parametric
CAD RG	.332	213	.000	.295	213	.000	Positive	Non-parametric
CAD YB	.255	213	.000	.570	213	.000	Positive	Non-parametric
Cone LL	.180	213	.000	.701	213	.000	Positive	Non-parametric
Cone LR	.166	213	.000	.735	213	.000	Positive	Non-parametric
Cone C	.331	213	.000	.328	213	.000	Positive	Non-parametric
Cone UR	.203	213	.000	.539	213	.000	Positive	Non-parametric
Cone UL	.193	213	.000	.696	213	.000	Positive	Non-parametric
Rod LL	.239	213	.000	.511	213	.000	Positive	Non-parametric
Rod LR	.167	213	.000	.706	213	.000	Positive	Non-parametric
Rod C	.121	213	.000	.808	213	.000	Positive	Non-parametric
Rod UR	.161	213	.000	.684	213	.000	Positive	Non-parametric
Rod UL	.328	213	.000	.304	213	.000	Positive	Non-parametric

Appendix E.2: Global significance testing

Table E-3: Global significance testing comparing all three groups using the Kruskal–Wallis test.

	H	df	P, Sig
CAD_RG	56.570	2	<.0001, Sig
CAD_YB	71.390	2	<.0001, Sig
ConeLL	70.955	2	<.0001, Sig
ConeLR	74.484	2	<.0001, Sig
ConeC	80.166	2	<.0001, Sig
ConeUR	51.796	2	<.0001, Sig
ConeUL	59.821	2	<.0001, Sig
RodLL	58.808	2	<.0001, Sig
RodLR	64.748	2	<.0001, Sig
RodC	52.729	2	<.0001, Sig
RodUR	57.352	2	<.0001, Sig
RodUL	60.466	2	<.0001, Sig
PhVA(+)	20.048	2	<.0001, Sig
PhVA(-)	21.846	2	<.0001, Sig
PhFCS(+)	19.197	2	<.0001, Sig
PhFCS(-)	28.440	2	<.0001, Sig
MeVA(+)	25.809	2	<.0001, Sig
MeVA(-)	14.932	2	.001, Sig
MeFCS(+)	9.664	2	.01, Sig
MeFCS(-)	7.553	2	.023, Sig

Note: (-) denotes negative contrast stimuli; (+) denotes positive contrast stimuli.