The Relationship between Diet and Tear Analysis in Routine Optometric Practice

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Summary

Dry eye syndrome is a common condition, regarded as a tear film abnormality which increases with age and causes symptoms such as dryness, irritation and grittiness. Its occurrence is often related to other health conditions such as diabetes.

Current treatments deal with the management of the tear film, and the conventional and common approach is instillation of lubricating eye drops or tear substitutes. These topical treatments generally treat the symptoms but are unable to resolve the underlying causes. It is therefore important to investigate the development of prevention strategies, and to investigate potential relationships between dietary factors and signs and symptoms of dry eye syndrome.

This thesis set out to investigate the relationships that might exist between dietary factors and signs and symptoms of dry eye syndrome. Two hundred participants were recruited from the patient base of an optometric practice in North Lincolnshire. An initial health and lifestyle questionnaire and food recall diary were completed by all participants. Results from an Ocular Surface Disease Index (OSDI) questionnaire and from clinical assessments made by the researcher were used as outcome measures.

The aim of the present study was to investigate the relationship between diet and the results were from clinical assessment of the tear film in routine optometric practice. Data was collected and statistical analysis was carried out on the results obtained. The findings were:

- There was a significant difference in OSDI score between males and females, showing that females had significantly higher OSDI scores than males.
- Those participants who consumed nuts had a significantly lower OSDI score, a significantly higher tear meniscus height (TMH), and significantly higher non-invasive tear break-up time (NITBUT) and Tear Break-Up Time (TBUT) than those who did not.
- Participants who consumed oily fish had a significantly higher TMH than those who did not.
- High OSDI scores were found to be significantly associated with lower consumption of Poly-unsaturated Fatty Acids (PUFAs), carbohydrate and calories.

Key words:

Clinical assessment; dry eye syndrome; essential fatty acids; nutrition; OSDI

Dedication

For Iqra, Hajra, Aaliya and Ruqaya

Acknowledgements

I would like to begin by thanking my supervisor, Dr. Hannah Bartlett, who regularly gave up her personal time to share her thoughts and give expert opinion. Her support, words of wisdom and encouragement made it a privilege to complete this thesis.

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I am particularly grateful to all my staff at O'Brien's for their help throughout, especially the practice manager Jane Palmer.

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Abbreviations

ALA	Alpha-Linolenic acid
BMI	Body Mass Index
DES	Dry Eye Syndrome
DHA	Docosahexaenoic Acid
EFAs	Essential Fatty Acids
EPA	Eicosapentaenoic Acid
FFQ	Food Frequency Questionnaire
GSL	General Sale List (without need of a prescription)
LASIK	Laser Assisted in Situ Keratomileusis
MGD	Meibomian Gland Dysfunction
NITBUT	Non-Invasive Tear Break-up Time
OCI	Ocular Comfort Index
OSDI	Ocular Surface Disease Index
OTC	Over-the-counter (without need of a prescription)
P	Pharmacy only (prescription required)
PUFAs	Polyunsaturated Fatty Acids
RDI	Recommended Daily Intake
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
TBUT	Tear Break-up Time
TMH	Tear Meniscus Height
USDA	US Department of Agriculture
VA	Visual Acuity

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Chapter 1: Literature Review

1.1 Background

A literature review was carried out to investigate the current knowledge of the relationship between diet and clinical tear analysis. The terms and words used for the searches to find articles relating to dry eye and diet included 'dry eye', 'essential fatty acids', 'omega 3 and 6', 'nutrition', 'Sjögren's syndrome', 'diet' and 'antioxidants'.

A review was carried out to find all relevant studies of the highest level of evidence, published, unpublished. Studies were assessed to produce an unbiased summary of the findings of articles including those published in peer-reviewed journals. Studies were identified and in the first stage using a computerised search of various databases including Pub Med, Science Direct and Ovid Medline, Web of Knowledge and Google Scholar. The aim was to identify relevant cross-sectional studies. In the second stage, copies of the articles were requested and where possible obtained. Bibliographies of the retrieved articles were manually searched. In the third and final stage, articles were reviewed and relevant information incorporated into this manuscript. UK based and International studies were included.

Study designs considered included in the review were:

- Double-blind studies
- Randomised controlled studies
- Observational studies
- Reports detailing the combined meta-analysis of various studies

There was no limit for the number of years within in which the studies had to have been conducted.

Once identified, duplicate studies and those involving inappropriate topics were removed. Animal studies were not considered. There was a language restriction when reading the studies as only ones published in English (including those translated) were considered. However, only nine relevant papers written in languages other than English were identified and so these were taken as unlikely to have significantly affected the outcome of the review

Wherever appropriate, the quality of the studies was assessed to establish a threshold of the quality available and gain an understanding of the relative strength and weakness of the body of evidence and distinguish among studies in terms of overall satisfaction. To do this, the following checklist had to be met:

1. Research question: Did the authors describe their goal in conducting this research and was it easy to understand what they were looking to find?
2. Study population: Did the authors describe the group of people from which the study participants were selected or recruited, using demographics, location, and time period?
3. Groups recruited from the same population and uniform eligibility criteria: Were the inclusion and exclusion criteria developed prior to recruitment or selection of the study population? Were the same underlying criteria used for all of the subjects involved?

4. Sample size justification: Did the authors present their reasons for selecting or recruiting the number of people included or analysed? Did they go further to note or discuss the statistical power of the study?
5. Sufficient timeframe to see an effect: Did the study allow enough time for a sufficient number of outcomes to occur or be observed.
6. Outcome measures: Were the outcomes defined in detail?
7. Statement regarding possible conflicts of interest for the authors: Was this mentioned in the study?

1.2 Results of review

Dry eye syndrome (DES) is a common condition, regarded as an abnormality to the tear film which frequently increases with age. It is thought to worsen through a sequence of four events: [1,2,3]

- Decreased tear production or increased tear film evaporation (increased tear osmolarity);
- Decreased conjunctival goblet-cell density;
- Increased corneal epithelial desquamation;
- Destabilisation of the cornea-tear interface.

DES may also arise from lid closure abnormalities or environmental conditions e.g. air conditioning, central heating and during tasks that require visual concentration e.g. interacting with a computer display.

The tear film is a transparent film that is known to have the following functions:[4,5]

- Mechanical—for the flushing of cellular debris from the cornea and conjunctival sac;
- Nutritional—it nourishes the avascular cornea;
- Bactericidal—its antimicrobial properties reduce the likelihood of corneal infection;
- Lubrication—ensuring there is smooth movement of the eyelids over the globe during the blink mechanism.

Dry eye syndrome has been defined by the National Eye Institute (NEI) as "a disorder of the tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort."[6] The National Eye Institutes' Dry Eye Workshop Subcommittee (DEWS) produced a definition of dry eye disease supported within a comprehensive classification framework which reflected the current understanding of the disease. The committee reviewed the definition and classification presented at the 1995 National Eye Institute (NEI)/Industry Dry Eye Workshop 2007, concluding: "Dry eye is a disorder of the tear film due to tear deficiency or excessive evaporation, which causes damage to the inter-palpebral ocular surface and is associated with symptoms of ocular discomfort." [7]

Dry eye syndrome can be divided into two types: aqueous deficient and evaporative.[5]

1. Aqueous deficient DES which can be further broken down to Sjögren's and non-Sjögren's dry eye. In the non-Sjögren's version, it can arise from an obstruction in the lacrimal gland resulting in increased tear osmolarity.[8]
2. Evaporative DES may arise due to a meibomian oil deficiency or lid inadequacies such as an incomplete blink or a low blink rate (inability to properly distribute tears).[9]

Current treatments usually deal with the management of the tear film, and the conventional and main approach to treating DES is instillation of lubricating eye drops or tear substitutes, although these do not last very long as topical treatments treat the symptoms and are unable to resolve the underlying cause.[3]

Dry eye syndrome can also be related to systemic health conditions such as diabetes mellitus, thyroid disease, rheumatoid arthritis and systemic lupus erythematosus.[6] It has also been shown to be related to systemic medications, such as diuretics, antihistamines, antidepressants, cholesterol lowering agents, beta-blockers and oral contraceptives.[7, 8] Patients suffering from DES complain of ocular symptoms such as dryness, grittiness, stinging, burning and general discomfort. Presenting signs often include bulbar conjunctival redness, corneal staining, reduced tear break up time and tear meniscus height.

1.3 Epidemiology

Epidemiological studies on dry eye syndrome suggest vast differences in prevalence. The difficulty in determining the prevalence and extent of DES has stemmed in part from limited understanding of the pathophysiology of dry eye. The Dry Eye Workshop Subcommittee reviewed major epidemiological studies of dry eye and demonstrated that the prevalence of dry eye ranged from 5 to 30 % of individuals aged over 50 years.[10] Definitions of DES have differed from one study to another with no consensus on criteria for diagnosis, making results difficult to compare.[10] There has also been a lack of standardised clinical testing protocols to diagnose DES, and in addition to this clinical signs show poor reliability resulting in under-diagnosis of the condition.[10] Patients often just live with the symptoms attributing them to getting older, though some will self-treat using products obtained over the counter (i.e., without prescription). Results from The Gallup Survey of DES Sufferers (2005), suggest that only one in four people with dry eye symptoms consult a primary health care professional.[11]

Prevalence of DES more than doubles after the age of 80 years compared to under 60 years and being female increases the risk of DES by nearly 50% with an estimated 3.23 million women and 1.68 million men aged over 50 years suffering from moderate-to-severe DES.[1] Increased age and being female, especially if peri- or post-menopausal, are risk factors for DES. Postmenopausal women may be the largest at-risk group: this is due to a decrease in hormonal levels leading to loss of anti-inflammatory protection and decreased lacrimal secretion.[10] It is therefore commonly accepted that the incidence of DES increases with age and is greater in women, especially around and after the menopause. A study on 926 subjects aged 40 years and older, found a higher prevalence of DES in women.[10] According to another study, women experienced a sharp increase in the prevalence of DES earlier than men, around the age of 45, roughly at the onset of menopause.[13,14] The prevalence of dry eye was reported to be 14.4% in 3,722 subjects aged 48 to 91 years and it was noted that the prevalence of the condition doubled after the age

of 59.[15] A review of several large studies, conducted by the Epidemiology Subcommittee of the 2007 NEI's Dry Eye Workshop, confirmed that the prevalence of DES can be up to 30% in people aged over 50 years.[12]

Large epidemiological studies conducted in the US to estimate the prevalence of DES in women have confirmed that the prevalence of the condition increases from approximately 5.7% in pre-menopausal women (aged under 50 years) to 9.8% in women aged over 75 years, concluding that DES is prevalent, affecting over 3.2 million American women middle-aged and older.[16] The same researchers estimated the prevalence for DES among US men in 2009: they concluded that prevalence of DES increased with age, from 3.9% among men 50–54 years old to 7.7% among men 80 years and older. They further estimated that the prevalence of DES was 1.68 million men aged 50 years or older with an expectation that DES would affect over 2.79 million US men by 2030.[17]Research conducted in a hospital environment revealed that the incidence of DES among ophthalmic outpatients was 0.46% with a male: female ratio of 1:1. Fifty seven percent of the patients were above 50 years of age.[94]

Dry eye syndrome has been known to occur following corneal surgery and is a known common complication of LASIK (Laser Assisted in Situ Keratomileusis).[18] This procedure is thought to disrupt both the dense sub-basal nerve plexus and stromal corneal nerves in the creation of the required anterior stromal flap and excimer laser ablation of the cornea.[19] There are complex interactions between the afferent sensory nerves of the ocular surface and the efferent autonomic nerves to the lacrimal gland that modulate both tear composition and secretion. Any factor that disrupts this relationship will lead to tear dysfunction and an increase in the concentration of the tear film (hyperosmolarity) which then leads to inflammation and apoptosis of the epithelium. The main proposed cause is therefore thought to be iatrogenic corneal nerve damage.[18, 19]

It has been shown that up to 10% of the non-contact lens wearing population who are under the age of 60 years have symptoms of dry eye,[20] although, paediatric contact lens wearers aged between 8 to 14 years have fewer complaints about dry eyes than adult contact lens wearers. This may be due to improved tear film, differences in reporting of symptoms, or modality of contact lens wear. For young females wearing contact lenses, researchers concluded that the osmolarity of their tears was not affected by contact lens use.[21] However, a survey conducted by US practitioners showed that up to 50% of the 35 million male and female contact lens wearers present to an eye care professional with dry eye symptoms, with 12-21% of soft contact lens patients having reduced their wearing time because of these symptoms, and that 6-9 % were so symptomatic that they were unable to wear lenses at all.[20]

The ocular surface and pre-corneal tear film have been investigated to see if they were influenced by the environment. The pre-corneal tear film was shown to be more influenced by climatic conditions than by atmospheric pollution.[96]

A study in India has revealed that overall the incidence of DES was higher amongst outdoor workers and people from rural areas, which may be due to the heat, low humidity, high temperature, direct hot air currents and dust, all of which are encountered in tropical climatic conditions and could have a drying effect on the pre-ocular tear film and the ocular surface.

These conditions, alone or in combination, may initiate or aggravate a dry eye. This study also revealed an association between DES and poor socioeconomic status, most probably due to a diet lacking green leafy vegetables and/or fruits and also vitamin A.[94]

Other researchers similarly concluded that exposure to wind, sunlight, high temperature, and air pollution was significantly related to dry eyes. Rural people and those with outdoor occupations in tropical climates are more exposed to environmental factors which can affect the tear film and ocular surface thus causing dry eye syndrome. [95]

1.4 Aetiology

Dry eye syndrome has a multifactorial and elusive aetiology and a wide variety of clinical signs and symptoms. There are two main aetiological hypotheses for categorising DES: aqueous-deficient or evaporative. [5, 6, 7, 9,100,101,102] In 2007, as recommended by DEWS, the scheme retained the two major classes of dry eye, as previously determined in the 1995 NEI/Industry Dry Eye Workshop classification.[103]

1.4.1 Aqueous-deficient DES

This is caused by a lack of aqueous tear secretion by the lacrimal glands. It results in hyperosmolarity of the tear film and an unstable tear film with desiccation of the ocular surface. It is often called aqueous deficient dry eye, or lacrimal insufficiency dry eye. Aqueous deficiency is a common accompaniment to auto-immune disorders such as rheumatoid arthritis, lupus and Sjogren syndrome which can be further classified as primary or secondary Sjögren syndrome. [5,6,7,9]

1. Primary Sjorgen is an auto-immune disorder in which the lacrimal and salivary glands are infiltrated by T-cells which are activated. The resulting symptoms include dry eye and dry mouth.[104]
2. Secondary Sjögren syndrome is associated with other autoimmune diseases such as rheumatoid arthritis.[23]

Non-Sjögren aqueous-deficient dry eye can result from lacrimal gland insufficiency and lacrimal duct obstruction.

1.4.2 Evaporative DES

This is known to have various causes: including meibomian gland disease, eyelid aperture disorders or lid/globe incongruity, blink disorders, and ocular surface disorders.[23] The most common cause is meibomian gland dysfunction (MGD); it is also often referred to a posterior blepharitis—which is a common condition of MGD.[24]

Meibomian gland dysfunction occurs when the lipid secretion required to control evaporation and maintain a normal tear film is abnormal, i.e. lipid layer deficiency. As MGD progresses, symptoms develop and lid margin signs, such as changes in meibum expressibility and quality. Lid margin redness may also become more visible.[25]

The International Workshop on Meibomian Gland Dysfunction proposed the classification of MGD into two major categories based on meibomian gland secretion: low-delivery states and high-delivery states. Low-delivery states are further classified as hyposecretory or obstructive, with cicatricial and non-cicatricial subcategories.[105] Hyposecretory MGD describes the condition

of decreased meibum delivery due to abnormalities in meibomian glands without remarkable obstruction. Obstructive MGD is due to terminal duct obstruction. In the cicatricial form, the duct orifices are dragged posteriorly into the mucosa, whereas, in non-cicatricial MGD, these orifices remain in their normal positions. High-delivery, hypersecretory MGD is characterised by the release of a large volume of lipid at the lid margin that becomes visible on application of pressure on to the tarsus during examination.[26] Deficiency of vitamin A, which is needed for maintaining the health of epithelial tissues, also appears to result in evaporative DES.[16]

Both aqueous-deficient and evaporative DES give rise to similar signs and symptoms and both types lead to increased evaporation and reduced tear film stability. Evaporative DES is generally regarded as the most common type although both forms can occur simultaneously in the most severe of cases.

1.5 Current treatment methods

1.5.1 Artificial tears

Current treatments usually deal with the management of the tear film, and remain the conventional and main approach to treating dry eye.[3] The exact mechanism of the treatment is difficult to identify as these artificial tear preparations do not recreate the function of the tear film, but do seem to have a lubrication effect.[27] The instillation of eye drops, sprays or tear substitutes reduces the tear osmolarity, improving the electrolyte balance and protecting the ocular surface. Lubricants are available without prescription, as general sale list (GSL) or over the counter (OTC) products, or pharmacy only (P) medicines requiring a prescription. This form of therapy gives only short term relief as topical treatments treat the immediate symptoms by lubrication but are unable to resolve the underlying cause.[3]

Although most tear supplements act solely as lubricants, other actions might include replacement of deficient tear constituents, dilution of pro-inflammatory substances, reduction of tear osmolarity,[27, 28] and protection against osmotic stress.[29]

The use of drops with high lubricity index has now become more common. For example, Systane drops by Alcon Laboratories have the lowest coefficient of friction and therefore the highest lubricity index value compared with other readily available preparations such as GenTeal by Novartis Ophthalmics, Refresh Tears and Refresh Endura by Allergan.[25]

A recent study was carried out to investigate the effect of a single-drop instillation of different lacrimal substitutes on tear film thickness assessed with optical coherence tomography in patients with mild to moderate dry eye disease.[97] The findings indicate that a single instillation of eye drops increases tear film thickness in patients with dry eye disease. However, the effect of multiple instillation and long-term use of artificial tears on tear film thickness warrants further investigation.

1.5.2 Nutrition and dietary intake

The various topical treatment modalities for DES only give only temporary relief from the symptoms, and this has prompted research into the development of prevention strategies. Nutrients are thought to be important in the maintenance of a physiologically normal tear film. Much of the research thus far has focused on polyunsaturated fatty acids (PUFAs) such as omega-3 essential fatty acids (EFAs) and omega-6 EFAs and how they are linked to the incidence

of dry eye. Generally, omega-3 EFAs can be found in walnuts and oily, cold-water dark fish such as tuna and salmon. Omega-6 EFAs are found in meat, corn oil and margarine.[22]

1.5.2.1 Essential fatty acids

Essential fatty acids (EFAs) are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. Essential fatty acids contain the carboxyl group (COOH) at one end and so are known as carboxylic acids.

The EFAs of particular relevance to ocular disease are:

1. omega-3 EFAs
2. omega-6 EFAs

The term omega, as it relates to fatty acids, refers to the terminal carbon atom farthest from the functional carboxylic acid group (–COOH). The designation of polyunsaturated fatty acids (PUFAs) as omega-3 EFAs, for example, defines the position of the first site of unsaturation relative to the omega end of that fatty acid. Thus, an omega-3 EFA such as α-linolenic acid (ALA), which contains three carbon-carbon double bonds (i.e. sites of unsaturation), has a site of unsaturation between the third and fourth carbons from the omega end, see Figure 1.1).

There are three major types of omega-3 EFA that are ingested in foods and used by the body: these are α-linolenic acid (ALA), see Figure 1.1, eicosapentaenoic acid (EPA), see Figure 1.2, and docosahexaenoic acid (DHA), see Figure 1.3. Once eaten, the body converts ALA to EPA and then to DHA. Generally, omega-3 EFAs are found in fish and fish oils, as well as seeds, oils, green leafy vegetables such as broccoli and spinach, nuts and beans.[25]

Omega-3 EFAs, such as ALA, EPA, and DHA. can be found in nature. Most of the ALA consumed in the diet comes from plant sources such as flaxseed, walnuts, pecans, hazelnuts, and kiwifruit.[34] The highest concentrations of EPA and DHA are found in cold water dark fishes such as salmon, tuna, and herring.[25]

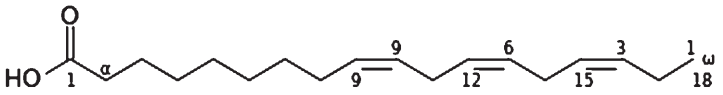

Figure 1.1—Chemical structure of alpha-linolenic acid (ALA)[30]

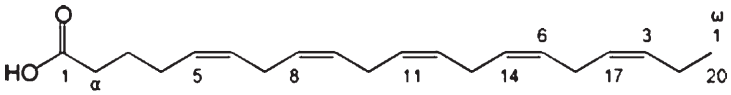

Figure 1.2—Chemical structure of eicosapentaenoic acid (EPA)[30]

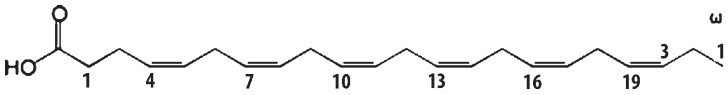

Figure 1.3—Chemical structure of docosahexaenoic acid (DHA)[30]

Most of the omega-6 EFAs consumed in the diet are from sources such as poultry, eggs, cereals

and whole-grain breads.[46] They are also found very commonly in oils such as: corn, peanut, safflower, rapeseed, sunflower, soybean, borage, and acai berry.[31] (see Figure 1.4).

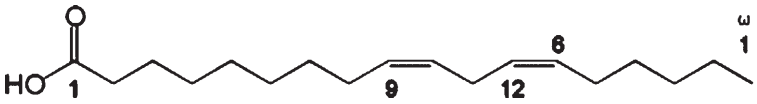

Figure 1.4—Chemical structure of linoleic acid, a common omega-6 fatty acid.[30]

Essential fatty acids cannot be produced within the body, but omega-3 EFAs and omega-6 EFAs are needed for bodily functioning. Essential fatty acids include linoleic acid (in the omega-6 EFA group) and alpha-linoleic acid (in the omega-3 EFA group). Both have a role in the maintenance of fluidity, flexibility, and permeability in the body.[24]

Current estimates of the dietary ratio between omega-3 EFAs and omega-6 EFAs in the western world are as low as 1:25. It has been recommended to the public that the ratio should be greater than this, ideally1: 4.39 However, the ratio is typically much lower (1:10-30) in a western diet due to greater consumption of meat and processed food.[35] If too much omega-6 EFA is ingested due to a diet high in processed meats and low in unprocessed oils and fish containing omega-3 EFA, the by-product of excess pro-inflammatory prostaglandins and too little anti-inflammatory prostaglandins may lead to dry eye. Over-consumption of omega-6 EFA can lead to heart disease, stroke and other degenerative diseases.[32] Hence, EFAs have been primarily studied with regard to cardiovascular disease and conditions such as rheumatoid arthritis, where omega-3 EFAs have been found to have anti-inflammatory properties.[25,26]

Omega-3 EFAs may alleviate dry eye symptoms by reducing inflammatory activity in the body possibly by altering the lipid profiles of the meibomian glands, while some components of the omega-3 EFAs are thought to stimulate aqueous tear secretion.[25] A large prospective, randomised, double-masked, placebo-controlled clinical trial of an omega-3 supplement for dry eye concluded there is evidence to suggest that certain dietary constituents, such as PUFAs, and particularly omega-3 EFAs, may be effective in dealing with the underlying causes of dry eye and reduce the risk.[27] Omega-6 EFAs may be useful in other ocular disease. A prospective study of dietary fat consumption found that diets high in omega-6 EFAs and low in omega-3 EFAs were associated with reduced chances of developing primary open angle glaucoma, in particular the normal-tension type, by making available omega-6 EFAs prostaglandins and therefore helping maintain intra-ocular pressure which does not harm the optic nerve.[25]

The relevance of unsaturated fatty acids in DES has been investigated. For example, sixteen patients suffering from dry eyes had a diet supplemented with unsaturated fatty acids during a period of one to four months, in addition to their existing dry eye therapy. The dietary supplement of omega-3 EFAs led to an improvement of symptoms and reduction of ocular surface staining.[25] It is understood that essential fatty acids are important for the production of the oily part of the tear film.[32] EFAs may enhance the lipid layer of the tear film, thus retarding evaporation,[33] although the use of essential fatty acids as a nutritional supplement is still regarded as a novel treatment for patients with DES.[34] There has been interest in the use of nutritional supplementation or dietary modification for the prevention and treatment of DES.[35]

A short study assessing the effect of oral omega-3 fatty acids on tear break-up time (TBUT), Schirmer's score and Ocular Surface Disease Index (OSDI) demonstrated that oral consumption of omega-3 EFA,180 mg EPA and 120 mg DHA twice daily for 30 days, is associated with a decrease in the rate of tear evaporation, an improvement in dry eye symptoms, and an increase in tear secretion, thus demonstrating that oral consumption of omega-3 EFAs has a definite role for DES as it is associated with a decrease in the rate of tear evaporation, an improvement in dry eye symptoms, and an increase in tear secretion.[42] This study was limited to 30 days. The results and conclusions seen here should be confirmed with a study over a longer duration.

Research in Singapore to evaluate the role of dietary supplementation of omega-3 EFA in DES was conducted and its response to intervention was monitored by routine tear function tests like Schirmer test, TBUT and Rose Bengal staining. Omega-3 EFAs were shown to have a definite role for DES.[41] A pilot, prospective, randomised, double-masked study over ninety days was conducted where patients with dry eye received a daily dose of fish oil.[28] This involved two patient visits: baseline and final. At these visits, patients completed the ocular surface disease index to score subjective symptoms, and had slit-lamp examinations—TBUT, corneal staining, Schirmer type I and fluorophotometry. The average tear production and tear volume, as indicated by Schirmer testing, was increased in the omega-3 group.[28] The observed improvement of dry eye relative to symptoms in this placebo-controlled randomised trial is an example of a very small sample size of subjects.

In a much larger sample size of subjects, Miljanović et al[39] assessed the diets of 32,470 women and also found that those with higher omega-3 fatty acids consumption had decreased risk for dry eye.

In a 90-day, placebo-controlled, double-masked study, patients were randomly assigned to receive a daily placebo or a daily capsule containing either 1000 milligrams (mg) of omega-3 EFAs in the form of flaxseed oil (TheraTears Nutrition) or 450 mg of omega-3 EFAs in the form of fish oil containing 450 mg of EPA. Results showed that 7% of symptomatic patients in the placebo group became asymptomatic while 70% on the omega-3 EFAs fish oil supplement became asymptomatic.[32] No trends were seen with the flaxseed oil. Although the results of this pilot study could not achieve statistical significance, with only 36 patients, 21 in the active group and 15 in the placebo group. The study suggests that the most promising end points for a larger clinical trial would include dry eye symptoms, Schirmer test results, and fluorophotometry.

Research has been carried to investigate the importance of nutrition and nutritional supplements in eye health and how oral nutritional supplementation alleviates the symptoms of DES. One study used 43 subjects randomised and subsequently followed for six months. There were two groups in the study. Group 1 (n = 23) was assigned to take two soft geltabs of a medical food supplement by mouth twice daily for six months. The four daily geltabs contained a total of 1 g of omega-3 EFAs derived from flaxseed oil and 500 mg of omega-6 EFAs derived from evening primrose oil. Group 2 (n = 20) was directed to take the medical food supplement in the same manner, along with topical cyclosporine, instilled twice daily during the last three months of the study. Subjects were evaluated at baseline, month 1, month 3, and month 6. Primary outcome measures included tear breakup time (TBUT), conjunctival staining, corneal staining,

and change in subjective symptoms. This study concluded that practitioners should at the very least consider omega-3 EFA supplementation.[36]

A study was carried out on 181 patients diagnosed with bilateral moderate dry eye who were already being treated with lacrimal substitutes. The patients were randomised in a double-blind international study to receive placebo or Nutrilarm (R) capsules (a combination of omega-3 EFA and omega-6 EFA), twice a day for 6 months. The study confirmed that oral administration of double supplementation dietary fatty acids present an additional therapeutic advantage in patients suffering from ocular dryness who were already being treated with lacrimal substitutes.[40] This study indicates that omega-3 supplementation induces improvement to the ocular surface with statistical significance ($P < 0.001$) in contrast to the placebo group ($P=0.250$). Of note, there was a lack in correlation between symptomatic improvement and the Schirmer score. This result was in contrast to the pilot study by Wojtowicz et al [39] in which there was increase in tear production and volume. The difference in findings between studies could be due to the small sample size in their study (n=36). The significant drift in the symptoms and TBUT scores in the omega-3 group suggest that dietary supplementation with omega-3 fatty acids improves the inherent stability of tear film as compared to tear production.[40]

Research confirmed that additional intake of nutritional supplementation with omega-3 fatty acids at an appropriate consumption ratio with omega-6 fatty acids was found to have a reduced incidence of dry eye due to its anti-inflammatory effects. Omega-3 fatty acids may be considered as an adjunct therapy to conventional tear substitutes.[43] Further research and clinical studies are necessary to validate the efficacy and safety of any nutritional supplements. There is currently no recommended daily allowance of any EFA, but companies producing such supplements suggest between one to three grams per day.

A study entitled The Women's Health Study investigated the relationship between diet and DES. It revealed that dry eye sufferers were more likely to have low levels of omega-3 EFAs in their diet, although concluding that further work is required to confirm the potential positive role of omega-3 EFAs in the prevention of DES.[47] A large epidemiological study conducted on 32,470 women aged 45 to 84 years, reported that those who consumed more omega-3 EFAs were less likely to report DES. Omega-3 EFA is thought to prevent DES by controlling inflammation in the tear gland and eye surface.[28] Results showed that women with the highest levels of omega-3 EFAs in their diets had a 20% reduced risk of dry eye compared with women with their lowest levels of omega-3 EFAs in their diet. Women who ate at least five servings of tuna per week had 68% reduced risk of dry eye compared with women who ate one serving per week. The research concluded that higher dietary intake of omega-3 EFA is associated with a decreased presence of DES in women.[28, 35]

It is reported that up to 25% of patients consulting eye care practitioners present with dry eye symptoms as well as up to 50% of the 35 million contact lens wearers in the USA.[44, 45, 46] Studies focusing on contact lens wearers and the importance of proper nutrition to optimise the ocular surface also concluded that essential omega-3 EFAs and omega-6 EFAs in diet showed improvement in contact lens patients with DES.[46]

In summary, the role of omega fatty acids has shown a reduced incidence of dry eye in tear production and secretion. [28, 39, 40, 41] Further evaluation with regards to the role of essential fatty acids is an important topic that would benefit from large, multicentre, randomised clinical trials to reach a conclusion about the positive effect of certain food types in the prevention and treatment of DES.

1.5.2.2 Antioxidants

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Some antioxidants are essential for the functioning of the human body: for example, vitamin A is an antioxidant and is an essential nutrient for humans because it cannot be synthesised within the body. Certain other antioxidants are not essential: for example, lutein which may be obtained from dietary sources.

Vitamin A is obtained from two sources:

- Preformed vitamin A from animal products such as milk, fish and liver.
- Provitamin A (carotene), from plant products such as green leafy vegetables, yellow fruit or red palm oil.[48]

Vitamin A is essential for the normal growth of and maintenance of the integrity of epithelial cells and tissues. In the absence of vitamin A, keratinisation of epithelial tissue occurs which could result in possible death of the tissue.[49]

Patel et al [49] assessed the tear stability of 60 healthy subjects and repeated the measurement 10 days later. Using a double-masked protocol, during the interim period, 66% of the subjects (the treatment group) took a commercially available daily dietary supplement and the remaining 33% acted as controls. Twenty of the treatment group took a daily dietary supplement consisting of vitamins (e.g. A, B1, B2, B6, E) and trace elements (e.g. calcium iron, manganese) (VisionACE®, Vitabiotics, London, UK), the other 20 subjects took vitamin C tablets. The tear stability of both treated groups increased. However, the multi-vitamin and trace element group demonstrated more consistent and individually predictable improvement. The tear stability of the control group individuals did not significantly change. Further study with a larger number of subjects over a longer period would be beneficial.

More recently, the same group investigated the effect of supplementation with vitamins C and E on ocular surface cytology specimens and clinical tear film parameters in 60 subjects with diabetes, again using vitamin C (1000 mg/day) and vitamin E (400 IU/day) for 10 days.[49] There was a statistically significant increase in goblet cell densities (50 to 59 cells/per field) after supplementation along with increased values for the Schirmer test, tear break up time and ocular ferning all reflecting an improved tear film quality. The researchers concluded that the tear stability of both treated groups increased and therefore supplementation with antioxidant vitamins C and E could play an important role in improving the ocular surface milieu.[51]

A cross-sectional study was carried out involving male patients seen in the Miami veterans affairs eye clinic. The patients completed a food frequency questionnaire and a dry eye questionnaire. They then underwent measurement of tear film parameters. The main outcome

measures included the association of vitamin D levels and DES. Higher vitamin D levels had a small but favourable effect on the symptoms of dry eye.[48] Research in Australia by Jalbert and colleagues suggested potential associations between diet and the health of the tear film. It is understood that malnutrition, protein deficiency and in particular vitamin A deficiency are extremely deleterious to the health of the eye. Vitamin A deficiency can cause xerophthalmia and keratomalacia so supplementation with oral vitamin A would be of clear benefit.[49]

Overall, the effectiveness and tolerability of dietary supplementation with a combination of omega-3 EFA and antioxidants in the treatment of DES have confirmed that dietary supplementation with a combination of omega-3 EFA and antioxidants is an effective treatment for DES.[52] More cross-sectional studies are needed to determine the indications for the use of these nutraceuticals, their required composition and dosage and to fully characterise how they modulate the tear film. [50]

1.5.2.3 Cholesterol

The leading study available at the time the present study was being carried out was produced in South Korea where elevated serum cholesterol was found to be associated with an increased risk of dry eye disease in women recruited to the fifth annual Korea National Health and Nutrition Examination Survey. Around 15% of the 3219 women in the study were clinically diagnosed with dry eye disease.[52] Results of this study highlight the significance of eye examinations and independent lipid profile monitoring in patients with dyslipidemia because of its possible correlation with DES progression.[53] Studies investigating the relationship between dry eye symptoms and daily intake of a range of dietary components measured using a three-day food diary showed that daily intake of cholesterol was significantly related to higher reported incidence of scratchiness symptoms in the cohort.[54]

To summarise, very few studies exist relating cholesterol and DES. The main study carried out in Singapore[54] shows results specific to the dietary habits of that population: therefore results and conclusions of this study needs to be backed up by studies from other parts of the world.

1.5.2.4 Hydration

Adequate daily water intake is recognised to be important for general health. Many would be aware of the recommendation to "drink six to eight glasses of water each day". [108] However, interestingly, there is a lack of rigorous scientific evidence to support this advice. The first published research carried out to identify if whole-body hydration plays an important role in DES hypothesised and confirmed that individuals classified as DES have higher tear osmolarity, indicating sub-optimal hydration, compared with those classified as not having DES.[55]

A small pilot study involving 29 participants with symptoms of dry eye, about three-quarters of participants reported a decrease in symptomatology after being advised to increase their daily water intake for two-weeks.[106] Research subsequently has compared changes in tear osmolarity using TearLab®. Results revealed tear osmolarity increased with dehydration.[56] These results were confirmed by other researchers looking to determine if tear fluid osmolarity changes due to hydration status during exercise and post exercise rehydration.[107] Tear osmolarity

decreased or remained approximately the same after rehydration for most subjects.[57] Sherwin et al hypothesised that hydration status may affect the eye in both healthy and disease states. The researchers performed a systematic review of the current evidence implicating clinical correlations of changes in hydration in major common eye diseases. Their findings suggested that systemic hydration status broadly affects a variety of ocular processes and disease states and therefore dehydration may be associated with development of DES.[58]

On the whole, later research confirms what was found in the first published research carried out to investigate if whole-body hydration plays an important role in DES.[55] Further research is needed to rigorously evaluate the value of altering hydration status to treat the signs and symptoms of DES.

1.6 Rationale and aim for this research study

Although not sight-threatening, DES is an ocular disorder that can severely reduce quality of life: ageing of the population means that DES is likely to become more common. Studies have shown that the degradation of ocular optical qualities related to DES is associated with visual impairments noticed during critical tasks such as driving. These impairments result from changes in aberration caused by changes in tear film.[72] It is important to investigate the development of prevention or reduction strategies. Large prevalence studies and studies relating dietary intake with dry eye have been conducted in other parts of the world but few studies and data are available for the U.K.

Differences will exist between countries with regard to dietary habits and preferences as well as socioeconomic, religious and environmental factors contributing to the different foods which are consumed by populations based in different parts of the world.

The subjects of the present study were based in a North Lincolnshire town in the U.K. and their dietary habits are likely to vary slightly from those of people in other areas of the U.K.

The aim of the present study was to investigate the relationship between diet and clinical assessment of the tear film in routine optometric practice. In the next chapter, the methods for the study will be presented.

Chapter 2: Methods

2.1 Aim

In Chapter 1, the literature pertaining to the aetiology and epidemiology of DES, and to the relationship between nutrition and DES, was presented. The present chapter describes the methods used in this study to assess a group of patients in a routine optometric practice in order to investigate the relationship between dietary intake and clinical assessment of DES.

2.2 Ethical approval

This study was approved by the Aston University Ethics Committee (ethics application number 367) and the work conforms to the tenets of the Declaration of Helsinki. A copy of the ethics form and letter from the University Ethics Committee can be found in Appendix 1.

This study involved recruitment of participants with and without the signs and symptoms of DES from the patient base of a clinical practice located in the United Kingdom. The nature of the study was explained to each participant in person by the researcher and an information sheet handed to each participant. Informed consent was obtained by explanation of the study and possible consequences of taking part. A copy of the subject information sheet and consent form was retained by the subject, this can be found in Appendix 2. All participants were informed that they were under no obligation to participate, and that they could withdraw at any time.

2.3 Subjects and recruitment

2.3.1 Inclusion and exclusion criteria

The aim was to explore relationships between DES symptoms and nutrition in a general sample of the population as found in an optometric practice. Participants were not selected on the basis of having, or not having, DES.

For inclusion participants had to:

1. Be over the age of 18
2. Agree to provide written informed consent.
3. Be available for one visit to the research centre.

Participants were excluded from the study if:

1. They had a history of any other anterior ocular pathology other than dry eye
2. They were taking medication affecting the ocular surface
3. They had undergone refractive surgery in the past
4. They had used eye drops within the 24 hours prior to data collection.

The analysis was controlled for smoking and contact lens wear.

In all, two hundred participants were recruited, all of whom were Caucasian. Participants were recruited from the staff and patients of a community optometric practice in a town in North East Lincolnshire, England over a period of 18 months. Advertisements requesting participants were placed in the waiting room of the practice. A copy of the advertisement can be found in Appendix 3.

2.4 Baseline data collection

2.4.1 Health and lifestyle questionnaire

An initial health and lifestyle questionnaire was completed by each participant. This provided information about general health, medication, Body Mass Index (BMI), nutritional supplementation, smoking history, ocular health and contact lens wear. A copy of the questionnaire can be found in Appendix 4.

2.4.2 Dietary Intake and nutritional software

There are several methods available to help assess the dietary intake of an individual. Food diaries can be used to assess current intake, as can 24-hour recalls, food frequency questionnaires (FFQ) or taking detailed dietary history. There is no gold standard in dietary assessment methods. All methods have their own strengths and weaknesses. The most common problem is unreliability because of misreporting.[59, 60] Food diaries and 24-hour dietary recall diaries are the most accurate methods when estimating mean intakes as they are the only methods that provide data on foods actually eaten. Both the food frequency questionnaire and the diet history are based on the long-term eating habits of the subject.

For this study, a 24-hour food recall diary was chosen, in preference to an FFQ, as FFQs have been found to result in an over-estimation of foods eaten less often, or a bias towards foods perceived as 'healthy' such as fruit and vegetables. There is some evidence that over-estimation increases with the length of the food list and therefore measurement error with FFQs is likely to be greater than that with other methods such as food diaries.[61]

A single 24-hour recall is also thought to be more useful to obtain a snap shot of dietary intake in a large group.[62, 63] National surveys have been known to use the 24-hour recall methods, mainly due to their high response rate and ability to obtain detailed information in a short time frame.[59] However, a single 24-hour recall may not be considered to be representative of habitual diet at an individual level: therefore repeated 24-hour recalls are becoming more popular to assess typical dietary habits.[62] A repeated 24-hour recall was performed over two non-consecutive days including one weekend day. This method increases the likelihood of capturing accurate habitual dietary habits and to permit estimation of within-person day-to-day variability. [59 62 63]

A high degree of cooperation and motivation from subjects, in particular from the elderly, is required as people can become easily fatigued and frustrated with long dietary history records which may be more problematic due to the limitations of memory.[64, 65] During the present study, this possibility was minimised by encouraging immediate input into a diary with exact detail such as 'two slices of thin sliced brown toast, thinly spread with butter'.

A copy of the food recall diary can be found in Appendix 5.

As mentioned earlier, the recall diary was completed over a 24-hours weekday and a 24-hours weekend day and, for the sake of consistency, the researcher interviewed all the participants in order to retrieve the information.

Nutritional software *a la calc* was chosen to extract the nutritional information gathered from the food diary. *A la calc* was easy to use and allowed the input of the information from the food diary in a simple but detailed manner. It produced nutritional results which were concise and easy

to extract. Each participant's food was entered into the software as a 'recipe', which allowed the software to extract forty nutritional characteristics and most essential nutrients.

A la calc was founded in 2011 in the UK by Richard Fong, Simon Funke and Kin Fong as a way to find the total nutrient content of a recipe by summing up the included ingredient nutrition content and accounting for weight lost in the cooking process of the food. The key target customers for *a la calc* are manufacturers, caterers, restaurants, cafés, sandwich makers and other food outlets. *A la calc* was chosen over other dietary analysis programmes because of the range of nutrients analysed, ease of use and compatibility with statistical analysis software, accessibility and cost.

The team behind the *a la calc* software confirmed that for accuracy and validity a la calc relies on three recognised nutritional databases for its calculations. These three databases contain thousands of ingredients in various states e.g. raw/cooked/frozen.and all use data that is updated regularly thus ensuring that calculated data from *a la calc* is always reliable and correct.

The three databases used within *a la calc* are:

1. A UK ingredient database maintained by McCance and Widdowson and officially approved by the relevant UK government organisations as the UK nutrient databank.
2. A US database data provided by the US Department of Agriculture (USDA): this is the official database for use in the US.
3. The third database is maintained by *a la calc* and is used for specific ingredients, including stabilisers, preservatives and flavour enhancers: this data is sourced from UK manufacturers of those products.

All *a la calc* calculations conform to the EC Directive 90/496/EEC which is used for professional nutritional labelling. The nutritional content is determined from the diet diary completely. The *a la calc* software uses a 'calculation' method to obtain nutrient value. For each ingredient in the recipe:

$$\text{Ingredient nutrient value} = \text{nutrient content of the ingredient} \times \text{weight of the ingredient}$$

$$\text{Recipe nutrient value} = \text{sum of all individual ingredient nutrient values}$$

Unless, the nutrient is water, in which case:

$$\text{Recipe nutrient value} = \left\{\begin{array}{c}\text{sum of all individual ingredient}\\ \text{nutrient values}\end{array}\right\} - \left\{\begin{array}{c}\text{total weight of all input ingredients}\\ \times \text{ weight lost in the cooking process}\end{array}\right\}$$

This total nutrient value is then scaled to a value per 100 grams.

The calculation assumes that all the weight lost in cooking is due to moisture which has evaporated.

The results obtained from the software were divided in two to average for one day as the data related to a two-day diary (one weekday and one weekend day). Brunner et al. confirmed that dietary analysis using the use of food diaries is reported to be well correlated with nutrient biomarker levels.[66]

An alternative to the 'calculated' method as used by *a la calc* would have been a 'lab analysis' method. However, the calculated method was preferred for the following reasons:

- Lab results are accurate to the portion of food sent in the sample. Calculated results are based upon average ingredient nutrient content.[109]
- Nutrient content for an ingredient can vary season to season or specimen to specimen. For example, a summer carrot may have higher nutritional content than a winter carrot due to temperature, heat and light changes.[110]
- Calculated methods cannot take into account physical deforming of nutrient molecules caused by extreme cooking methods.[111]
- Both calculated and lab results are accepted by the UK Food Standards Agency for showing on retail packs the nutritional content of food. Benefits of the a *la calc* software included the ability to extract information about the consumption of antioxidants such as vitamins A, C, E and zeaxanthin, and of carbohydrates (some of which are sugars), cholesterol and calories. The limitations of this software include inability to extract omega-3 or omega-6 nutrient values from polyunsaturated fatty acids. To allow for this, it was noted whether the participant had consumed nuts or oily fish.

2.4.3 Dry eye questionnaire

There are a number of other dry eye questionnaires available, including:

- Ocular Surface Disease Index (OSDI) [63, 64]
- McMonnies Dry Eye Index [63, 64]
- Ocular Comfort Index (OCI) [64]
- National Eye Institute Visual Functioning Questionnaire [65]

For this study, it was determined that the dry eye questionnaire to be employed should meet the following criteria:

1. The questionnaire must have been validated.[63,64]
2. The questionnaire must be readily available and appropriate for practitioners.[65]

The OSDI questionnaire was chosen as studies have shown it to be a valid and reliable instrument for measuring the severity of dry eye disease.[67] This questionnaire uses a 12-item, 5-category Likert scale that investigates symptoms, triggers and consequences of dry eye.[68] It utilises the twelve questions to assess the level of discomfort and how dry eye syndrome interferes with daily living tasks. Five of the twelve questions relate to ocular symptoms, four to functional tasks and three to environmental triggers.

It is generally accepted that an OSDI score of around 30 or over is necessary for diagnosis of severe DES.[70] Studies have shown that the OSDI correlated significantly with the McMonnies questionnaire, the National Eye Institute Visual Functioning Questionnaire, the physical component summary score of the Short Form-12, patient perception of symptoms, and artificial tear usage.[69] It is suggested that the OSDI score is proportional to symptom intensity.[69]

For the sake of consistency, the researcher interviewed all the participants for the completion of the questionnaire. A copy of the OSDI questionnaire can be found in Appendix 6.

2.5 Clinical data collection

Subjects were assessed by the researcher using clinical techniques found in routine optometric practice. The results were recorded and a copy of the data collection form can be found in Appendix 7.

A strict protocol was developed for data collection. The assessment was conducted in a stable, air-conditioned environment where the temperature in the consulting room was maintained at 20 degrees centigrade. Subjects remained in this environment between measurements as recommended in the International Dry Eye Workshop report.[71]

2.5.1 Non-Invasive tear break-up time (NITBUT)

There is concern that the presence of fluorescein in the tear film will destabilise the tears and for this reason the tear film was measured non-invasively without first instilling fluorescein.[72,73,74] This type of tear film measurement is referred to as non-invasive tear break-up time (NITBUT). It should however be noted that changes in the tear meniscus curvature has been observed even with this minimally invasive technique confirming that it is easy to induce minor degrees of reflex tearing, even during delayed blinking.[75] A Bausch & Lomb (one-position) variable doubling keratometer was used to observe the distortion and/or break up time of the keratometer mire (a reflected image of a keratometer grid). The researcher focused and viewed the crisp mires and then recorded the time for the mire image to break up. For a reliable clinical estimate of the NITBUT, it is recommended taking three measures of NITBUT: this can then be confidently used as a representative value of tear stability time.[112] Three consecutive readings were evaluated and the median noted. This is an established clinical test that is routinely used in optometric and ophthalmological practice.

2.5.2 Tear meniscus height (TMH)

A normal pre-ocular tear film should be continuous over the cornea, conjunctiva and lid margin. The height of the tear meniscus can give an indication of tear volume. This was measured by a slit-lamp biomicroscope with a graticule in 0.05 mm units at the centre of the lower lid margin. This measurement is normally a one-time snapshot during the inter-blink period. Although the tear system is dynamic and obviously, the tear meniscus can keep changing from time to time and is affected by many factors, like glare and heating.[113] The slit was positioned horizontal to the lower lid with indirect illumination, to exclude these invasive factors. The TMH was measured directly below the pupil centre.

Tear meniscus height is classified:

good: greater than 0.2 mm;
normal: 0.2 mm;
poor: less than 0.2 mm.[76]

Many studies have demonstrated that there is good correlation between TMH and symptoms of dryness.[77,78,79]

In the present study, three consecutive readings were taken and the median noted. This is an established clinical test that is routinely used in optometric and ophthalmological practice.

A study by Mainstone et al found that TMH has strong correlations with non-invasive breakup time and is the most powerful predictor of tear film insufficiency. The conclusion was that TMH is a useful alternative to existing tests for dry eye.[114] However, the main limitation of tear meniscus assessment remains the lack of universally accepted normative data and cut-off values for normal eyes. Mean TMH values range from 0.12 ± 0.04 to 0.46 ± 0.17 mm in healthy eyes and from 0.13 ± 0.07 to 0.24 ± 0.09 in the case of dry eye.[115]

2.5.3 Tear break-up time (TBUT)

Tear stability is routinely assessed in clinical practice to aid in the diagnosis of DES. Assessing the tear film can be difficult as the tear film is transparent: fluorescein dye can be introduced into the tears to make observation of the tear film break up easier.

In the present study, fluorescein stain was applied into the temporal lower palpebral conjunctiva of the eye by a moist Fluoret—a paper strip impregnated with approximately 1mg fluorescein sodium Ph.Eur. When wet with a sterile drop the resulting orange dye fluoresces green when excited by blue light. Following instillation the patient was asked to blink a few times to spread the dye over the surface of the eye. The uniform green film was observed and the time recorded for black patches to start to appear, as these are signs of the tear film breaking up. The tear film was assessed using a narrow slit, full-beam slit-lamp biomicroscope. The number of seconds taken for the tear film to break up was recorded. The break up was seen as diffuse dark patches which appeared within the spread of fluorescein over the cornea.

This technique of assessing stability of the tear film is sometimes thought of being controversial, even though it is widely used. It can be made more reliably when conducted using a full-beam observation of the cornea is used to scan the cornea with a narrow slit in making the measurements.[116] Three consecutive readings were evaluated and the median noted. This is an established clinical test that is routinely used in optometric practice: in fact, fluorescein staining for assessing tear break-up time is the most frequently used test for DES diagnosis.[80] Despite the possibility of wide variation in TBUT among individual subjects, there is general agreement that a TBUT shorter than 10 seconds reflects tear film instability and a TBUT shorter than 5 seconds is a definite marker of dry eye.[81]

This technique is the most widely used clinical test for assessing tear instability and therefore measurement of TBUT.[117,118] One study has concluded the reproducibility of TBUT is high in the general population but significantly greater in patients with dry eyes.[82] In contrast, another study has recognised that TBUT has poor accuracy and so best conducted with other clinical measures.[119] The main characteristic of the test that may contribute to its poor performance is the variability in the volume of fluorescein instilled.[120, 121] The original tear volume is a minute volume, and addition of even a tiny amount of fluorescein solution significantly disturbs native tear dynamics. A study showed that moistened fluorescein strips can deliver an inconsistent minute volume of fluorescein to the ocular surface, which, in turn, directly affects the TBUT measurement obtained.[121] However, Finnemore et al showed that when wet with a sterile drop, a fluorescein strip is designed to deliver a relatively accurate but very limited dose of from 0.5 ml

to 1.0 ml of liquid to the surface of the cornea, thus minimising variability.[122] This may explain why, overall, studies that use fluorescein strips, which deliver a minimal volume of fluorescein, result in a relatively reliable and repeatable reading.[123,124]

Several questions about TBUT are still unanswered and warrant further study: to determine which method of fluorescein delivery produces the most reliable results for repeatability and sensitivity/specificity in detecting dry eye.[123]

2.5.4 Corneal and conjunctival staining

In patients with DES, one or all of the corneal or conjunctival surfaces or the intracellular surfaces becomes compromised.[83] Staining agents allow these changes to be viewed. Corneal staining is believed to occur when fluorescein enters damaged epithelial cells.[84 85] Some degree of staining is found in up to 79% of corneas in healthy non contact lens wearing patients.[85] This form of staining is a measure of epithelial integrity and is widely used in clinical and research settings.

Grading scales are now used extensively in optometric practice for a variety of purposes including the anterior eye examination. This tool can now be considered as an expected norm in practice. The incorporation of grading scales is advocated for good optometric clinical practice.

Various clinical grading scales were considered including the original CCLRU rebranded IER and more recently published under the name of the Brien Holden Vision Institute or BHVI, and Efron grading scales. The latter consists of a series of artist-rendered depictions of 16 different conditions, while the former comprises photographs of six conditions, two of which are presented in multiple manifestations. The Efron grading scales were chosen for this study and according to Professor Nathan Efron, who developed these scales, the advantage of using an artist-rendered (versus photographic) grading scales is that the desired level of change can be depicted, with all other factors being kept constant, thus avoiding potentially confounding artefacts and allowing artistic licence to add clarity.[125] The primary design criteria in developing the Efron grading scales were simplicity and ease of use by clinicians. Complications are illustrated in five stages in severity (from 0 to 4), with traffic light colour banding from green (normal) to red (severe). The artist-rendered systems generally afford lower grading estimates and better grading reliability than the photographic systems.[86, 87]

Efron grading scales are validated for clinical use and show a linear change between grades. Practitioners can expect to use them with average 95% confidence limits of ±1.2 grading scale units.[86]

In this study, the cornea and the conjunctiva were assessed using fluorescein stain when measuring TBUT: Efron scales were used to record staining by type, depth and extent.

2.5.5 Protocol for data collection

1. Consent was taken by the researcher and a unique identification code created.
2. All data was collected by the researcher.
3. A health and lifestyle questionnaire was completed by the subject.
4. The subject was interviewed by the researcher for the food diary recall.
5. The researcher carried out the collection of clinical data a minimum of ten minutes after subject arrived to allow for acclimatisation to the indoor environment:
    - 1.1 Eye chosen for assessment
    - 1.2 Vision measured in chosen eye
    - 1.3 Visual acuity measured in chosen eye
    - 1.4 Non invasive tests carried out:
        - 1.4.1 NITBUT measured.
        - 1.4.2 TMH measured.
    - 1.5 Invasive Tests carried out:
        - 1.5.1 TBUT measured.
        - 1.5.2 Assessment of fluorescein corneal staining.
        - 1.5.3 Assessment of fluorescein conjunctival staining.
    - 1.6 The researcher interviewed the subject for the OSDI questionnaire

2.6 Data entry and analysis

Data from the health and lifestyle questionnaire, OSDI, *A la calc* and the clinical assessment were entered into Microsoft Excel v12.0 (Microsoft Office 2007, Microsoft Corporation, Washington, USA). The data were then exported for statistical analysis into SPSS v23.0 (IBM SPSS 23 software (IBM Corporation, New York, USA).

Each data set was checked for normality using the Kolmogorov-Smirnov test statistic which asseses the normality of distribution of the data. Where found to be normally distributed (significance value less than 0.05), the appropriate parametric test was used for statistical analysis. Where non-normally distributed, the appropriate non-parametric equivalent tests were used.

2.6.1 Sample size

Sample size data for comparison between groups has been calculated based on published mean and standard deviation tear film data [88] and shown in Table 2.1.

The means and standard deviations are taken from a publication by Lira et al [88]. The difference to detect values were determined to keep the values as small as possible to reflect realistic differences between groups. The difference in values as a percentage of the mean values are all between 10 and 20 %.

	TMH (mm)	NIBUT (sec)	TBUT (sec)
Mean / Standard Deviation	0.30 / 0.14	6.58 / 2.62	6.68 / 1.99
Difference to detect	0.06	1.5	1.5
Effect size (Difference to detect / SD)	0.35	0.57	0.75
Sample size (32 / effect size^2)	174	100	57

Table 2.1

Sample size data for comparison between groups

The maximum number of participants calculated was 174 for TMH. The recruitment target was set at 200 participants to allow for drop out.

Although considered controversial by some researchers, post-hoc sample sizes were carried out using mean and standard deviation data from the data collected in this study, and are included here for comparison. These sample sizes are shown in Table 2.2

	NITBUT (sec)	TBUT (sec)	TMH (mm)	Total corneal staining	Total conjunctival staining	OSDI
Mean / Standard Deviation	4.08 / 1.79	3.68 / 1.72	0.38 / 0.13	2.32 / 3.67	10.04 / 4.57	24.01 / 20.01
Difference to detect	1.50	1.50	0.06	2.40	2.40	10.00
Effect size (difference to detect / SD)	0.84	0.87	0.38	0.65	0.53	0.50
Sample size (32 / effect size^2)	46	42	179	75	116	128

Table 2.2

Post-hoc sample size data

2.7 Summary

In this chapter the methods for the cross-sectional study of the relationship between clinical evaluation, OSDI and dietary intake have been described. Chapter 3 will report on the baseline characteristics of the cohort.

Chapter 3: Descriptive and subgroup analysis

This chapter will outline the characteristics of the cohort and also examine differences between sub-groups and outcome measures.

3.1 Descriptive statistics

Two hundred participants were recruited all of whom were white British. Pre-existing signs and symptoms of DES were not a requirement for participation. The mean age of the subjects was 56 years (mean ± sd 56 ± 19, range 21 to 89 years). The median age was 60 years. One hundred and fourteen of the participants were female (57%), 15 were smokers (7.5%), 17 were contact lens wearers (8.5%).

As mentioned in the previous chapter, a TBUT of less than 5 seconds is generally considered indicative of DES,[81] and the mean TBUT for this cohort was 3.68 seconds (median 3 seconds). It was found that 168 subjects (84%) were found to have TBUT of less than 5 seconds. Conversely, a TMH greater than 0.2 mm is considered 'good', [76] and this cohort had a mean THM of 0.38 mm (median 0.3mm). Only two subjects from the 200 (1%) were found to have THM less than 0.2mm. An OSDI score of over 30 is generally needed to diagnose DES[70] and Table 3.1,below, reveals the mean OSDI score of the whole group to be 24.01 (median 16.67). It was found that 85 subjects (43%) had an OSDI score over 30.

The mean intake of PUFA for the group was 16.63 g which is comparable to the recommended daily intake (RDI) 16 g.[126,127] The mean intake of other nutrients such as saturated fat, carbohydrates, vitamin C, vitamin E, and cholesterol were all lower than the recommended daily intake (RDI). Details of the characteristics of the whole cohort alongside what is regarded as normal values and RDI can be found in Table 3.1 (next page), with graphical representation in Figure 3.1, page 35.

Whole group	Mean / Standard Deviation	Median	Normal Values	Recommended Daily Intake
Age (years)	56.2 / 19.9	60.00		
Height (metres)	1.7 / 0.2	1.70		
Weight (kg)	70.7 / 14.5	70.9		
BMI	25.05 / 7.49	24.10		
Vision (logmar)	0.45 / 0.37	0.50		
VA (logmar)	0.07 / 0.12	0		
NITBUT (seconds)	4.1 / 1.8	4.00	10	
TMH (mm)	0.4 / 0.1	0.30	0.2	
TBUT(seconds)	3.7 / 1.7	3.00	10	
TOTAL Corneal staining score	2.32 / 3.67	1.00		
TOTAL Conjunctival staining score	10.04 / 4.57	10.00		
Total OSDI score	24.01 / 20.01	16.67		
Polyunsaturated fatty acids (g)	16.63 / 8.80	15.50		16
Energy (kcal)	1251.07 / 470.73	1273.00		2500 †
Saturated Fat (g)	7.74 / 3.90	7.00		20.0
Carbohydrates (g)	59.69 / 40.36	47.00		275
Vitamin C (mg)	48.95 / 54.89	31.70		60
Vitamin E (mg)	5.21 / 3.59	3.80		15
Cholesterol (mg)	161.88 / 99.12	157.80		300

† Average requirement suggested by the European Food Safety Authority for males and females aged between 30 and 79 years.[151]

Table 3.1

Mean, standard deviation and median values for anthropometric, dry eye and dietary factors for the whole cohort, alongside RDI.[126 127] (n=200)

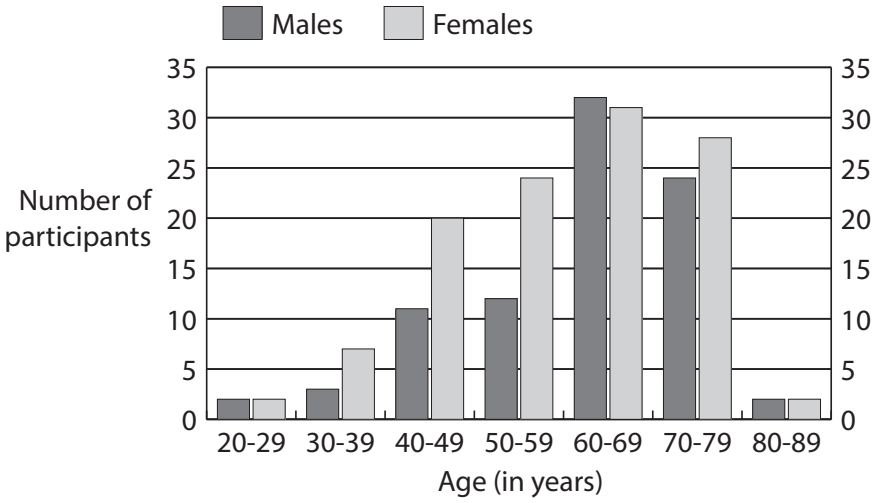

Figure 3.1
Age distribution of participants.

3.2 Age subgroups

The cohort was divided into subgroups for two age groups: participants aged 50 years and under and those over 50 years. Initial results revealed a lower mean NITBUT and TMH in the over 50 years subgroup. See Tables 3.2, below, and 3.3, next page.

50 years and under	Mean / Standard Deviation	Median	Normal Values	Recommended Daily Intake
Height (metres)	1.7 / 0.2	1.70		
Weight (kg)	71.7 / 15.1	70.00		
BMI	25.02 / 7.35	25.00		
Vision (logmar)	0.45 / 0.37	0.50		
VA (logmar)	0.08 / 0.13	0		
NITBUT (seconds)	4.2 / 1.9	4.00		
TMH (mm)	0.4 / 0.1	0.40	10	
TBUT(seconds)	3.6 / 1.6	3.00	0.2	
TOTAL corneal staining score	2.47 / 3.88	2.00		
TOTAL conjunctival staining score	10.12 / 5.24	10.00	10	
Total OSDI score	23.74 / 22.44	23.00		
Polyunsaturated fatty acids (g)	18.28 / 9.65	18.00		16
Energy (kcal)	1314.64 / 488.06	1301.00		2500 †
Saturated Fat (g)	7.79 / 3.72	7.00		20.0
Carbohydrates (g)	60.93 / 44.40	58.00		275
Vitamin C (mg)	46.83 / 57.07	46.00		60
Vitamin E (mg)	5.22 / 3.65	5.00		15
Cholesterol (mg)	168.99 / 91.43	160.00		300

† Average requirement suggested by the European Food Safety Authority for males and females aged between 30 and 79 years.[151]

Table 3.2

Mean, standard deviation and median values for dry eye and dietary factors for the 50 years and under sub-group, alongside RDI.
(n = 78)

Over 50 years	Mean / Standard Deviation	Median	Normal Values	Recommended Daily Intake
Height (metres)	1.7 / 0.2	1.70		
Weight (kg)	70.1 / 14.2	70.90		
BMI	25.16 / 7.61	24.10		
Vision (logmar)	0.46 / 0.37	0.50		
VA (logmar)	0.07 / 0.1	0		
NITBUT (seconds)	4.0 / 1.8	4.00	10	
TMH (mm)	0.4 / 0.1	0.30	0.2	
TBUT (seconds)	3.7 / 1.8	3.00		
TOTAL corneal staining score	2.22 / 3.54	1.00	10	
TOTAL conjunctival staining score	9.99 / 4.12	10.00		
Total OSDI score	24.18 / 18.33	16.67		
Polyunsaturated fatty acids (g)	15.58 / 8/07	15.50		6
Energy (kcal)	1210.42 / 456.7	1273.00		2500 †
Saturated fat (g)	7.71 / 4.03	7.00		20.0
Carbohydrates (g)	58.90 / 37.7	47.00		275
Vitamin C (mg)	50.31 / 53.64	31.70		60
Vitamin E (mg)	5.20 / 3.57	3.80		15
Cholesterol (mg)	157.33 / 103.85	157.80		300

† Average requirement suggested by the European Food Safety Authority for males and females aged between 30 and 79 years.[151]

Table 3.3

Mean, standard deviation and median values for dry eye and dietary factors for the over 50 years sub-group alongside RDI.[126,127]
(n = 122)

3.2.1 Statistical analysis

The difference between the independent sub-groups and the outcome measures was statistically assessed. According to the Kolmogorov-Smirnov statistic, none of the variables in the cohort were found to be normally distributed (significance value less than 0.05). For this reason the Mann-Whitney U Test was then used to investigate the difference in dry eye outcomes between the two age category subgroups.

The analysis revealed that there was no statistically significant difference in the dry eye outcome measures and between the age in the two sub-groups. This is likely to be due to the mean age of the subjects 50 years and under being 48.62 and over 50 being 58.18. These two age categories had a difference in means of only 10 years. As a result, there was not a significant difference in the dry eye outcome measures between the two age groups as the ages themselves did not differ enough from the cohort. Lifestyle factors such as differences in the smoking or contact lens wearing habits between the two groups may have had an effect on the results as 90% of 50 years and under and 94% of over 50 years did not smoke. Also, 92% of 50 years and under and 91% of over 50 years did not wear contact lenses. See Table 3.4, below.

	50 years and under			Over 50 years				
	N	Mean / Standard Deviation	Median	N	Mean / Standard Deviation	Median	Z	p
Age		48.62 / 17.92	48.00		58.18 / 19.20	59.00	– 0.21	0.83
OSDI score	78	23.74 / 22.44	23.00	122	24.18 / 18.33	16.67	– 0.77	0.44
TMH (mm)	78	0.40 / 0.13	0.40	122	0.38 / 0.13	0.30	– 0.94	0.35
NITBUT (seconds)	78	4.22 / 1.85	4.00	122	3.99 / 1.75	4.00	– 0.72	0.47
TBUT (seconds)	78	3.60 / 1.56	3.00	122	3.73 / 1.81	3.00	– 0.03	0.98
Total corneal staining	78	2.47 / 3.88	2.00	122	2.22 / 3.54	1.00	– 0.45	0.65
Total conjunctival staining	78	10.12 / 5.24	10.00	122	9.99 / 4.12	10.00	– 0.54	0.59

Table 3.4

Z and p values for relationships between the sub-groups 50 years and under and over 50 years and dry eye outcomes.

3.3 Male and female subgroups

The cohort was divided into male and female subgroups for statistical analysis. When divided into the subgroups, the lower mean NITBUT, TMH and TBUT in the female subgroup revealed a greater indication of DES than for the male subgroup. This was also evident in the OSDI score, which was greater in females than males. See Table 3.5 and Table 3.6, together with Figures 3.2, 3.3 and 3.4 on page 40.

Males	**Mean / Standard Deviation**	**Median**
Age (years)	56.0 / 21.4	60.00
Height (metres)	1.7 / 0.2	1.70
Weight (kg)	70.5 / 14.2	70.90
BMI	24.95 / 7.53	24.10
Vision (logmar)	0.46 / 0.36	0.50
VA (logmar)	0.06 / 0.10	0
NITBUT (seconds)	4.3 / 1.9	4.00
TMH (mm)	0.4 / 0.1	0.30
TBUT(seconds)	3.9 / 1.8	3.00
TOTAL corneal staining score	2.17 / 3.49	2.00
TOTAL conjunctival staining score	9.74 / 4.62	10.00
Total OSDI score	21.65 / 21.22	16.67
Polyunsaturated fatty acids (g)	17.42 / 9.39	18.00
Energy (kcal)	1293.99 / 494.1	1273.00
Saturated Fat (g)	7.92 / 3.78	8.00
Carbohydrates (g)	59.61 / 37.45	60.00
Vitamin C (mg)	45.44 / 45.75	31.70
Vitamin E (mg)	5.22 / 3.47	3.70
Cholesterol (mg)	171.55 / 108.30	157.60

Table 3.5

Mean, standard deviation and median values for dry eye and dietary factors for males.
(N=86)

Females	Mean / Standard Deviation	Median
Age (years)	56.3 / 18.8	60.00
Height (metres)	1.7 / 0.2	1.70
Weight (kg)	69.6 / 14.71	70.90
BMI	25.12 / 7.49	24.10
Vision (logmar)	0.45 / 0.37	0.50
VA (logmar)	0.08 / 0.13	1.00
NITBUT (seconds)	3.9 / 1.7	3.00
TMH (mm)	0.4 / 0.1	0.30
TBUT (seconds)	3.5 / 1.6	3.00
TOTAL corneal staining score	2.43 / 3.82	2.00
TOTAL conjunctival staining score	10.26 / 4.54	10.00
Total OSDI score	25.77 / 18.96	16.67
Polyunsaturated fatty acids (g)	16.04 / 8.32	15.50
Energy (kcal)	1218.68 / 451.8	1273.00
Saturated Fat (g)	7.60 / 4.0	7.00
Carbohydrates (g)	59.76 / 42.59	60.00
Vitamin C (mg)	51.60 / 60.93	50.00
Vitamin E (mg)	5.20 / 3.7	5.00
Cholesterol (mg)	154.58 / 91.4	157.80

Table 3.6

Mean, standard deviation and median values for dry eye and dietary factors for females.
(N = 114)

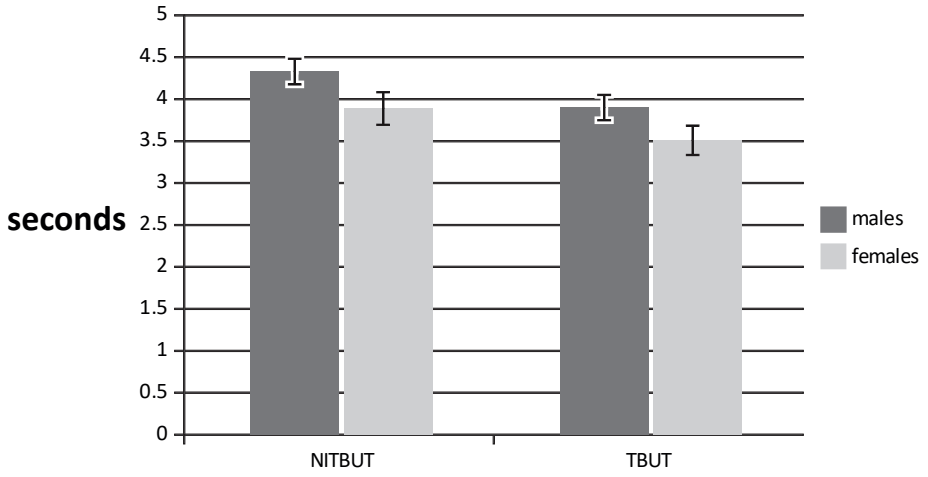

Figure 3.2
NITBUT and TBUT mean timings for males and females.

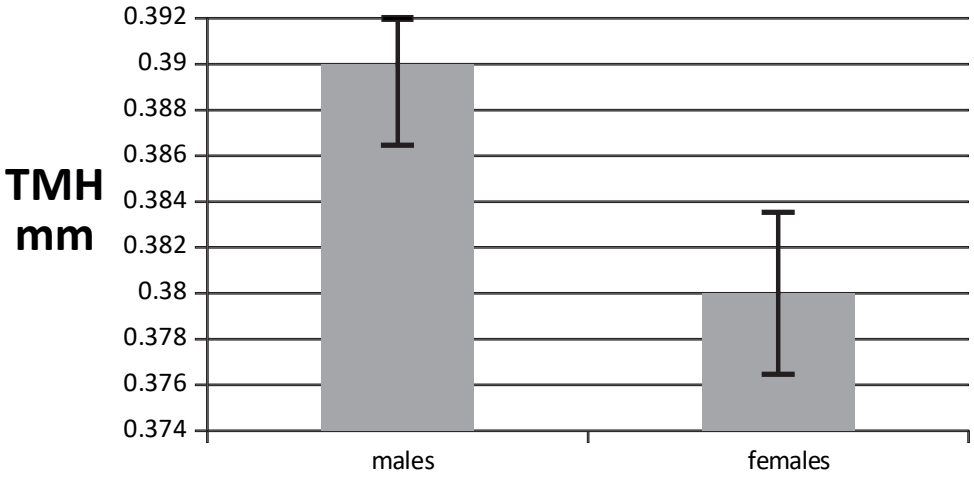

Figure 3.3
Mean TMH values for males and females.

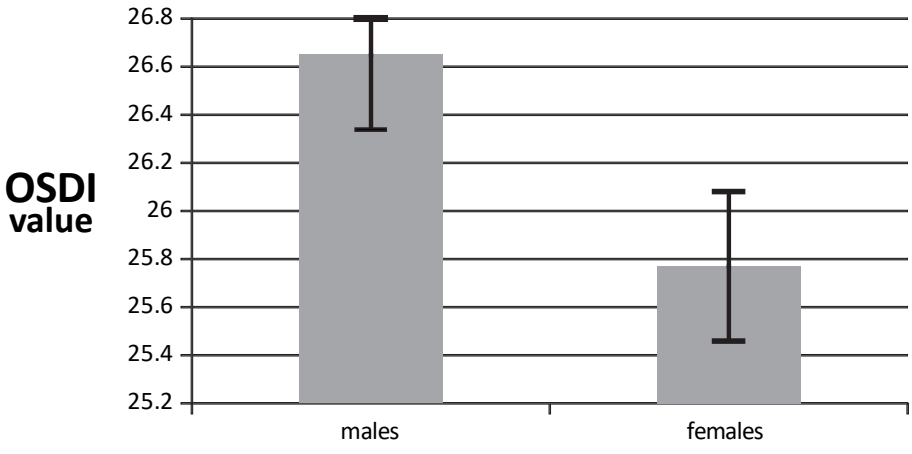

Figure 3.4
Mean OSDI values for males and females.

3.3.1 Subgroup analysis

The difference between the independent sub-groups and the outcome measures was statistically assessed. According to the Kolmogorov-Smirnov statistic, none of the variables in the cohort were found to be normally distributed (significance value less than 0.05). For this reason The Mann-Whitney U Test was then used to investigate the difference in dry eye outcomes between the male and female independent groups.

Using the Mann-Whitney U Test (significance value less than 0.05) there was a statistically significant difference in OSDI score. Females had statistically significant higher OSDI score ($Z = -2.23$, $p = 0.03$) with a small effect size using the Cohen (1988) criteria ($r = 0.16$). There was no statistically significant difference seen between the other dry eye outcomes and the two sub-groups. There was no significant difference between age and smoking prevalence between the two gender groups. The results are shown in Table 3.7.

	Males			Females				
	N	Mean / Standard Deviation	Median	N	Mean / Standard Deviation	Median	Z	p
50 years and under	35	33.4 / 9.11	32	43	35.44 / 8.17	35	1.08	0.31
Over 50 years	51	71.55 / 10.74	71	71	68.89 / 10.15	67	-1.54	0.11
Smokers	8	51.25 / 20.35	46	7	49.14 / 12.90	47	-1.29	0.14
Non-smokers	78	56.51 / 21.53	60.5	107	56.74 / 19.09	61	1.14	0.22
OSDI score	86	21.65 / 21.22	16.67	114	25.77 / 18.96	16.67	– 2.23	0.03
TMH (mm)	86	0.39 / 0.13	0.40	114	0.38 / 0.14	0.40	– 1.08	0.28
NITBUT (seconds)	86	4.33 / 1.85	4.00	114	3.89 / 1.72	4.00	– 1.77	0.08
TBUT (seconds)	86	3.90 / 1.80	4.00	114	3.51 / 1.64	3.00	– 1.46	0.15
Total corneal staining	86	2.17 / 3.49	2.00	114	2.43 / 3.82	2.00	– 0.48	0.63
Total conjunctival staining	86	9.74 / 4.62	10.00	114	10.26 / 4.54	10.00	– 1.03	0.30

Table 3.7

Z and p values for relationships between gender and dry eye outcomes.

3.4 Smokers and non-smokers subgroups

The cohort was divided into smokers and non-smokers subgroups for statistical analysis. The results showed there was a higher OSDI score for the smokers subgroup compared to non-smokers. See Tables 3.8, below, and 3.9, next page, together with Figure 3.5, page 45.

Smokers	**Mean / Standard Deviation**	**Median**
Age (years)	50.27 / 16.7	50.00
Height (metres)	1.7 / 0.3	1.70
Weight (kg)	73.6 / 16.6	70.90
BMI	25.29 / 7.91	25.00
Vision (logmar)	0.80 / 0.30	0.80
VA (logmar)	0.11 / 0.16	0
NITBUT (seconds)	4.1 / 1.6	4.00
TMH (mm)	0.4 / 0.1	0.40
TBUT (seconds)	3.7 / 1.7	3.00
TOTAL corneal staining score	1.53 / 1.85	1.00
TOTAL conjunctival staining score	9.80 / 5.58	10.00
Total OSDI score	30.85 / 28.59	30.00
Polyunsaturated fatty acids (g)	17.55 / 7.24	17.00
Energy (kcal)	1295.87 / 528.48	1273.00
Saturated Fat (g)	6.77 / 3.61	7.00
Carbohydrates (g)	70.26 / 64.98	70.00
Vitamin C (mg)	70.27 / 81.89	70.00
Vitamin E (mg)	4.83 / 3.32	5.00
Cholesterol (mg)	169.07 / 132.86	170.00

Table 3.8

Mean, standard deviation and median values for dry eye and dietary factors for the smokers sub-group.
(N = 15)

Non-smokers	Mean / Standard Deviation	Median
Age (years)	56.6 / 20.1	60.00
Height (metres)	1.7 / 0.2	1.70
Weight (kg)	70.5 / 14.4	70.90
BMI	25.03 / 7.48	24.10
Vision (logmar)	0.43 / 0.36	0.50
VA (logmar)	0.07 / 0.12	0
NITBUT (seconds)	4.1 / 1.9	4.00
TMH (mm)	0.4 / 0.1	0.30
TBUT (seconds)	3.7 / 1.7	3.00
TOTAL corneal staining score	2.38 / 3.78	2.00
TOTAL conjunctival staining score	10.06 / 4.50	10.00
Total OSDI score	23.44 / 19.13	16.67
Polyunsaturated fatty acids (g)	16.56 / 8.92	16.00
Energy (kcal)	1247.43 / 467.15	1273.00
Saturated Fat (g)	7.82 / 3.92	7.00
Carbohydrates (g)	58.84 / 37.83	58.00
Vitamin C (mg)	47.22 / 54.02	47.00
Vitamin E (mg)	5.24 / 3.62	5.00
Cholesterol (mg)	161.29 / 96.32	160.00

Table 3.9
Mean, standard deviation and median values for dry eye and dietary factors for the non-smokers sub-group.
(N = 185)

3.4.1 Smokers and non-smokers

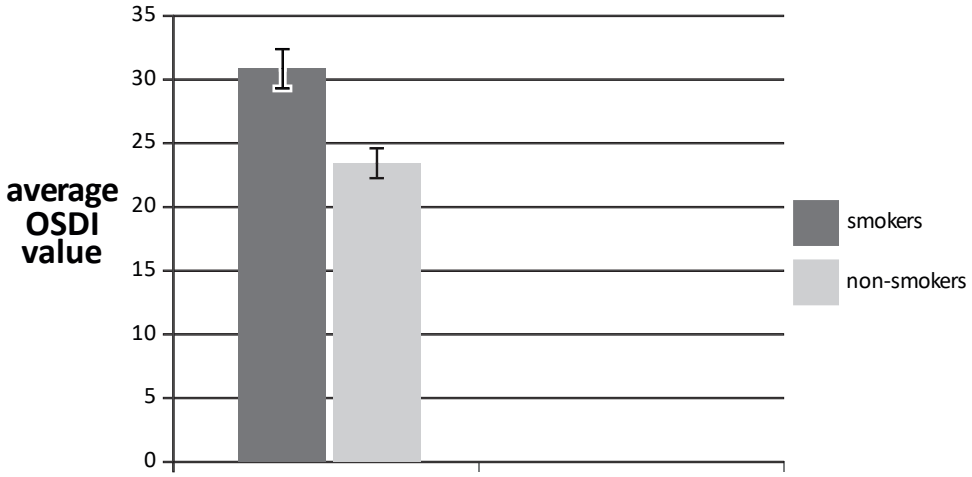

Figure 3.5
Mean OSDI values for smokers and non-smokers.

The difference between the independent sub-groups and the outcome measures was statistically assessed. According to the Kolmogorov-Smirnov statistic, none of the variables in the cohort were found to be normally distributed (significance value less than 0.05). For this reason The Mann-Whitney U Test was then used to investigate the difference between the smokers and non-smokers independent groups with the dry eye outcomes. There was no statistically significant difference in the dry eye outcomes between smokers and non-smokers. However, there was a statistically significant difference between the lifestyle habit of smoking and non-smoking in both males and females, (Mann-Whitney U Test significance value less than 0.05). This significant difference would justify the following analysis on a reduced cohort to age and gender match in section 3.4.2. The results are shown in Table 3.10, next page.

	Smokers			Non-smokers				
	N	Mean / Standard Deviation	Median	N	Mean / Standard Deviation	Median	Z	p
Males	8	51.27 / 16.72	47	78	56.51 / 21.53	60.5	0.88	0.04
Females	7	49.14 / 12.90	47	107	56.74 / 19.09	61	0.76	0.03
50 years and under	70	34.19 / 8.82	33.5	8	37.5 / 6.16	36.5	0.34	0.67
Over 50 years	7	64.86 / 11.94	58	115	70.31 / 10.32	69	0.29	0.90
OSDI score	15	30.85 / 28.59	30.00	185	23.44 / 19.13	16.67	−0.61	0.54
TMH (mm)	15	0.39 / 0.13	0.40	185	0.38 / 0.13	0.30	−0.28	0.78
NITBUT (seconds)	15	4.13 / 1.55	4.00	185	4.08 / 1.81	4.00	−0.46	0.65
TBUT (seconds)	15	3.67 / 1.68	3.00	185	3.68 / 1.73	3.00	−0.10	0.92
Total corneal staining	15	1.53 / 1.85	1.00	185	2.38 / 3.78	2.00	−0.31	0.76
Total conjunctival staining	15	9.80 / 5.58	10.00	185	10.06 / 4.50	10.00	−0.60	0.56

Table 3.10

Z and p values for relationships between smokers, non-smokers and dry eye outcomes.

3.4.2 Age and gender-matched smokers and non-smokers

The cohort was also separated into the sub-group of age and gender-matched smokers and non- smokers. As mentioned earlier, according to the Kolmogorov-Smirnov statistic, none of the variables in the cohort were found to be normally distributed (significance value less than 0.05). For this reason, The Mann-Whitney U Test was then used to investigate the difference between these two independent groups with the dry eye outcomes. As they were age matched, it was also statistically confirmed as there was no statistically significant difference in the ages (P = 0.98). There was also no statistically significant difference seen and in the dry eye outcomes between age and gender matched smokers and non- smokers. The results are shown in Table 3.11.

	Smokers			Non-smokers				
	N	Mean / Standard Deviation	Median	N	Mean / Standard Deviation	Median	Z	p
Age		55.61 / 18.88	55.00		55.11 / 18.11	55.00	–0.03	0.98
OSDI score	15	30.85 / 28.59	30.00	15	26.33 / 21.10	16.33	–0.58	0.48
TMH (mm)	15	0.39 / 0.13	0.40	15	0.39 / 0.13	0.40	–0.32	0.64
NITBUT (seconds)	15	4.13 / .55	4.00	15	4.01 / 1.62	4.00	–0.54	0.69
TBUT (seconds)	15	3.67 / 1.68	3.00	15	3.69 / 1.71	3.00	–0.11	0.88
Total corneal staining	15	1.53 / 1.85	1.00	15	2.16 / 3.66	2.00	–0.28	0.71
Total conjunctival staining	15	9.80 / 5.58	10.00	15	10.11 / 4.33	10.00	–0.48	0.51

Table 3.11
***Z* and p values for relationships between age and gender matched smokers, non-smokers and dry eye outcomes.**

3.5 Summary

Of this sample of 200 participants, the mean OSDI score was 24 (median 17), which is well below the cut-off for diagnosis of DES. The proportion of participants with an OSDI score of over 30 was 43%.

When comparing males and females, smokers and non-smokers, and those aged 50 years and below and those aged over 50 years, the only significant difference was that females had a significantly higher OSDI score than males (26 compared with 22). This gender difference is

supported by other studies highlighted in Chapter 1, which reported higher incidence of DES in perimenopausal women.[10,13,14,16,17]

The fact that no difference was found between smokers and non-smokers is counter-intuitive, but could be explained by the fact that only 15 of the participant smoked. However, these results held even when the smoking participants were compared with a group of 15 age- and gender-matched controls.

There was no significant difference between the sub-categories for age. The participants who were aged 50 years and under when compared with the sub-group over the age of 50 years showed no statistical significance. This counter-intuitive result could be explained by the fact the average ages for the two categories were very similar.

Chapter 4: The relationship between clinical methods of tear film evaluation

In the previous chapter, the baseline characteristics of the study cohort and subgroups were discussed. In this chapter, relationships that exist between the outcome measures will be explored.

4.1 Background

Previous studies have reported correlations between different measures of tear analysis. For example, the tear stability in young Nigerian adults was measured using both TBUT and NIBUT. Forty five subjects aged 20 to 30 years were selected from among the students of University of Benin, Edo State. The TBUT values were comparable to the NIBUT values: these results showed that tear film stability can be confidently evaluated by either technique.[89]

In another study the NITBUT was shown to be a more physiological method to assess tear film stability in patients with tear film disorders and the values measured by NITBUT were highly correlated with those measured by the TBUT.[90]

Correlation between OSDI and TBUT was explored when 68 patients admitted to the Ophthalmology Polyclinic of the Dumlupinar University in Turkey between December 2005 and April 2006 were studied when evaluating the OSDI for the diagnosis of DES. The OSDI questionnaire was completed before the routine examination and then the TBUT test was carried out. There was a significant inverse correlation between the OSDI and TBUT test scores ($r = -0.296$, $p = 0.014$). The conclusion was that OSDI is a standardised method which can easily be performed to evaluate symptoms to support the diagnosis of dry eye syndrome alongside tear tests such as TBUT.[91]

Similarly, the diagnostic values of the TBUT tests and OSDI in DES was assessed with thirty-five employees of the Istanbul Ümraniye Training and Research Hospital. All participants completed the OSDI. Following routine examination, the TBUT was undertaken and outcomes were compared. Investigators found that there was a significant inverse correlation between the OSDI and TBUT ($r = -0.385$, $p = 0.022$). The researchers concluded that the OSDI questionnaire, used together with the TBUT, is an easily performed test and may be of benefit in supporting the diagnosis of DES.[92]

The other tear tests used in this study have been studied before to help improve predictive ability for the development of dry eye symptoms. Tear meniscus height and NIBUT were significantly, but moderately related to OSDI scores ($r > 0.31$, $p < 0.05$).[93]

4.2 Methods

As described in Chapter 2, the subjects were assessed by the researcher using clinical techniques found in routine optometric practice.

4.3 Results

The baseline data were presented in tables in Chapter 3.

According to the Kolmogorov-Smirnov statistic, all the variables from the outcome measures were found not to be normally distributed (significance value less than 0.05). For this reason, a Spearman correlation was used to explore the strength of the relationships between them.

Analysis confirmed a statistically significant, weak negative correlation between OSDI scores and TBUT (rho = – 0.235, p = 0.001) and OSDI scores with NITBUT (rho = – 0.165, p = 0.02). NIBUT showed a strong positive correlation with TBUT (rho = 0.878, p = 0.0001) and a weak positive one with TMH (rho = 0.268, p = 0.0001). TMH showed a weak positive correlation with TBUT (rho = 0.225, p = 0.001). Results are seen as scatter plots in figures 4.1 to 4.5.

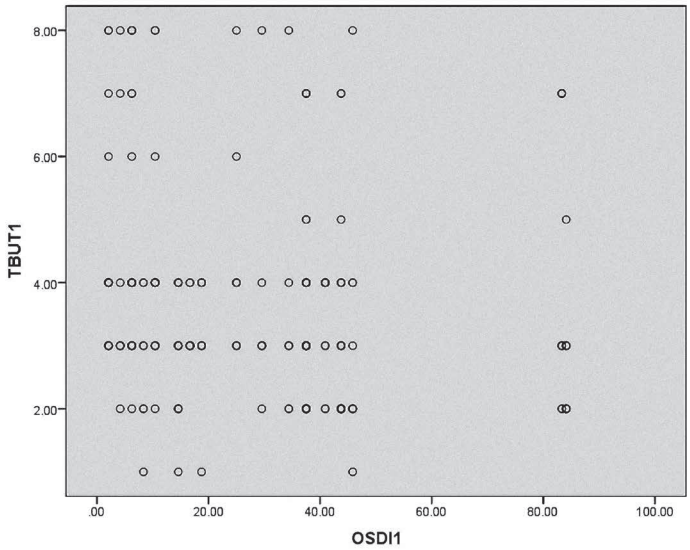

Figure 4.1—Scatter plot showing a weak negative correlation between OSDI score and TBUT.

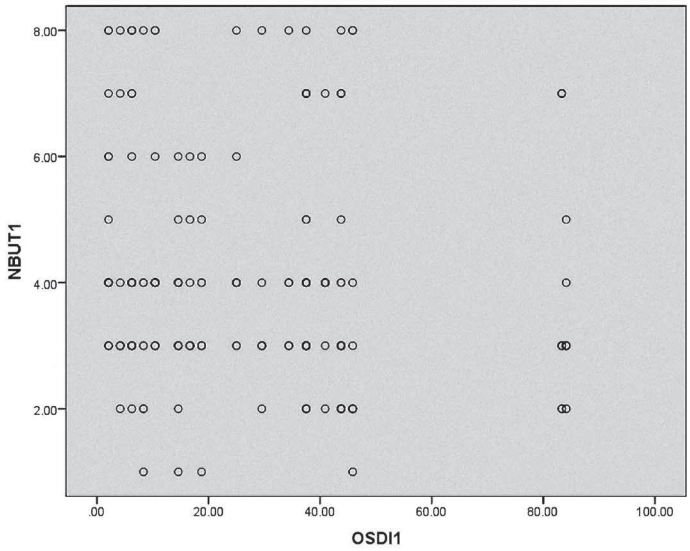

Figure 4.2—Scatter plot showing a weak negative correlation between OSDI score and NITBUT.

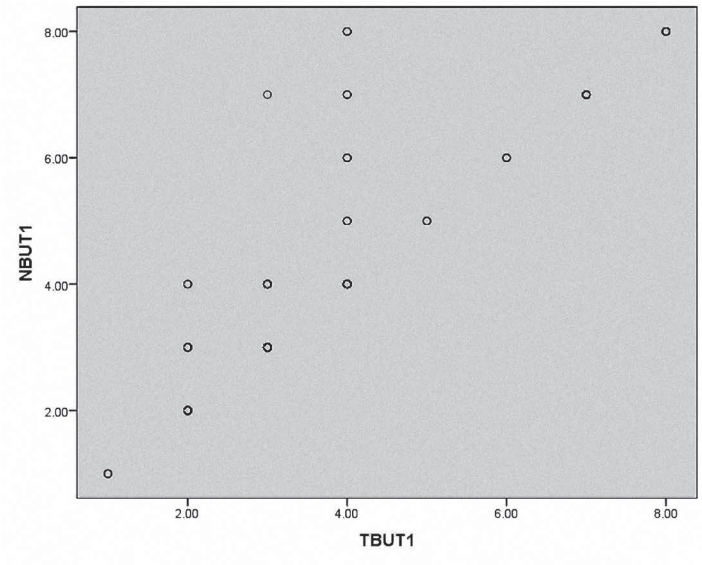

Figure 4.3—Scatter plot showing a strong positive correlation between TBUT and NITBUT.

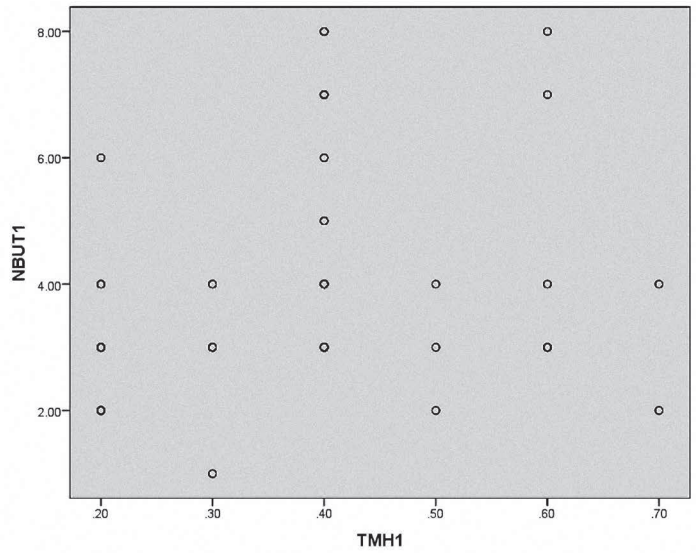

Figure 4.4—Scatter plot showing a weak positive correlation between TMH and NITBUT.

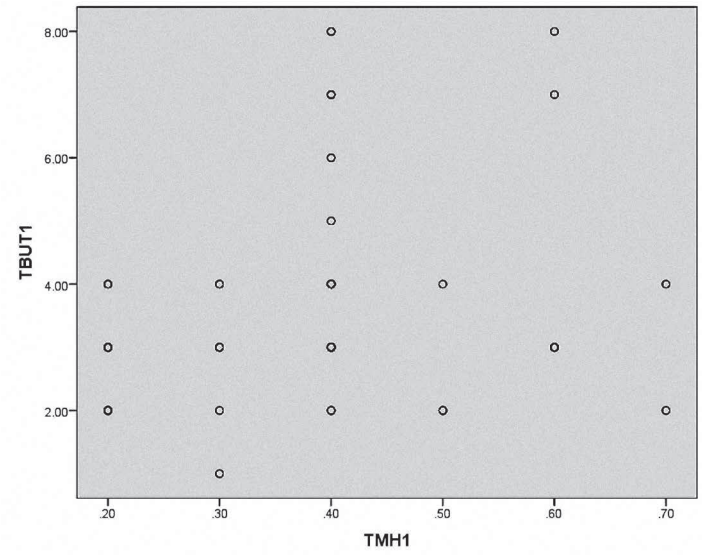

Figure 4.5—Scatter plot showing a weak positive correlation between TMH and TBUT

4.4 Logistic Regression

Of the 200 subjects of this study, 85 had an OSDI score of over 30 and were therefore diagnosed as having dry eye syndrome.

The data was transformed and a logistic regression was performed to see which of the different clinical parameters best predict severe dry eye as diagnosed using the OSDI score (over 30). The model contained two independent variables (TBUT and NITBUT). The full model containing these two predictors was statistically significant χ^2 (2, N = 2 00) = 69.10, $p < 0.001$.

The strongest predictor of dry eye was TBUT, recording an odds ratio of 8.11. This indicated that respondents who had a TBUT of less than 5 seconds were over 8 times more likely to suffer from dry eye syndrome than respondents with a TBUT greater than 5 seconds.

4.5 Summary

The negative correlations between OSDI scores and TBUT revealed a rho = – 0.235 and p = 0.001 demonstrating a weak strength of correlation. TBUT was also the strongest predictor of dry eye (OSDI score over 30). This was consistent with findings in another study.[92] In comparison, the negative correlation between OSDI score and NITBUT was weaker showing rho = – 0.165, p = 0.02. Again, this was a similar finding to that from a previous study. [93]

As found in other studies,[89,90] NITBUT was found to correlate well with TBUT with a strong positive correlation (rho = 0.878, $p < 0.001$): suggesting that tear film stability can be confidently evaluated by either of the techniques and therefore confirming that alongside the OSDI score NITBUT can easily be performed to evaluate symptoms to support the diagnosis of dry eye syndrome.

This study shows that TMH correlated significantly and similarly with both NITBUT and TBUT. Although there was an inverse correlation between TMH and OSDI score, this was not statistically significant. Other studies have found a statistically significant, but moderate, correlation between TMH and OSDI.[93] The key findings in this chapter are consistent with other studies, in particular studies where Pult et al[93] and Ozcura et al[91] concluded that deriving an OSDI score should be a standardised procedure to evaluate symptoms of and support the diagnosis and correlation with dry eye syndrome.[90,91,92,93] TBUT was the best predictor of dry eye syndrome, although as found in previous studies,[91,92] the low agreement found between subjective symptoms and objective tests reflects difficulties in making a diagnosis of dry eye as although the parameters are statistically shown to be related, the correlation is not perfect therefore suggesting there is a need to perform both objective and subjective tests because they provide different information.

Chapter 5: Analysis of relationships between tear film analysis and diet

5.1 Non-parametric statistical analysis

According to the Kolmogorov-Smirnov statistic, all the variables were found not to be normally distributed (significance value less than 0.05). For this reason, Spearman correlation was used to explore the strength of the relationship between the outcome measures of dry eye disease and dietary factors extracted from the dietary software.

The following nutritional dietary factors were extracted from the *a la calc* software as they these nutrients have been linked with DES in previous studies, as discussed in Chapter 1.

Polyunsaturated fatty acids (PUFAs encompassing EFAs)

Carbohydrates

Vitamin C

Vitamin E

Cholesterol

Calories

Saturated fat

Statistical analysis on the whole group concluded that high OSDI scores were significantly associated with lower consumption of polyunsaturated fatty acids showing a weak positive correlation (rho = 0.21, $p < 0.001$), a weak negative correlation with carbohydrate intake (rho = – 0.25, $p < 0.001$), cholesterol intake (rho = – 0.16, $p = 0.03$), calories (rho = – 0.33, $p < 0.001$) and saturated fat intake (rho = – 0.16, $p = 0.03$). Greater TMH was associated with higher consumption of cholesterol showing a weak positive correlation (rho = 0.18, $p = 0.01$). Results are seen in scatter plots Figures 5.1 to 5.5.

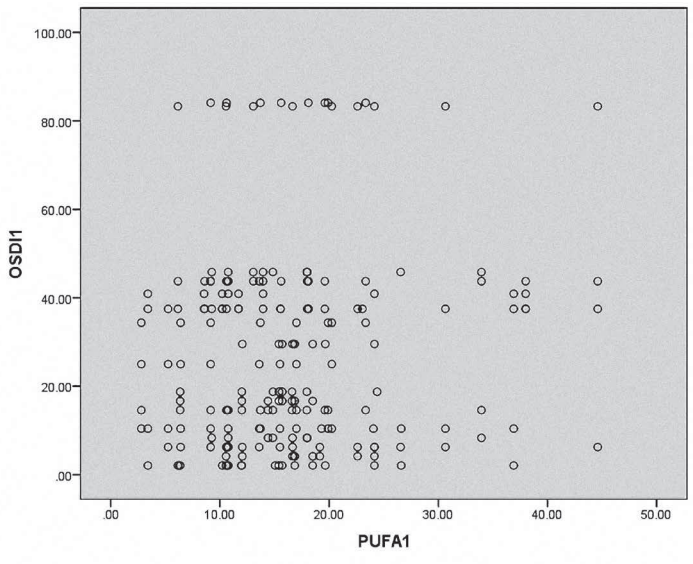

Figure 5.1—Scatter plot showing a weak negative correlation between PUFA intake and OSDI score.

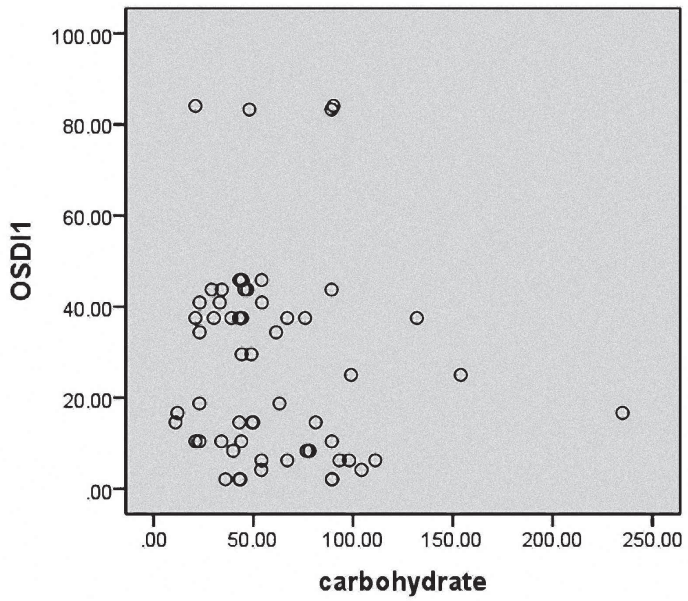

Figure 5.2—Scatter plot showing a weak negative correlation between carbohydrate intake and OSDI score.

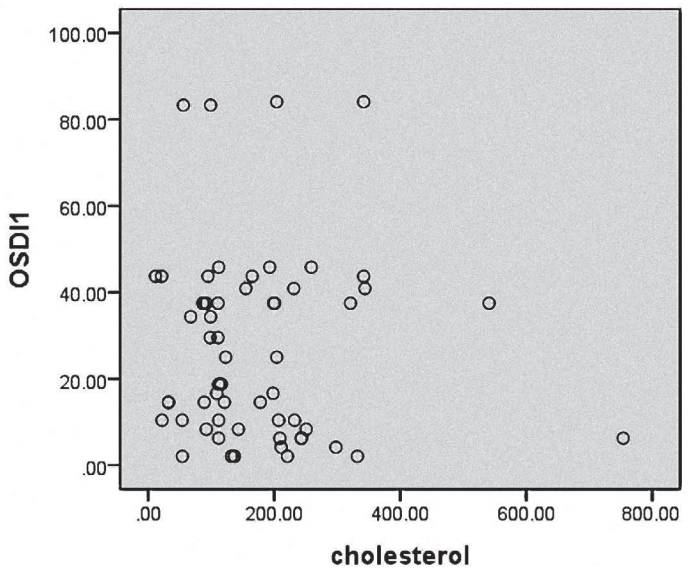

Figure 5.3—Scatter plot showing a weak negative correlation between cholesterol intake and OSDI score.

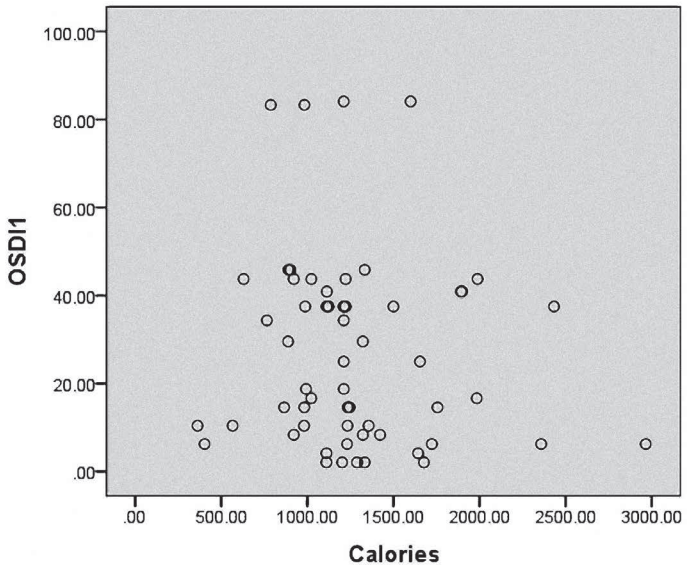

Figure 5.4—Scatter plot showing relationship between calorie intake and OSDI.

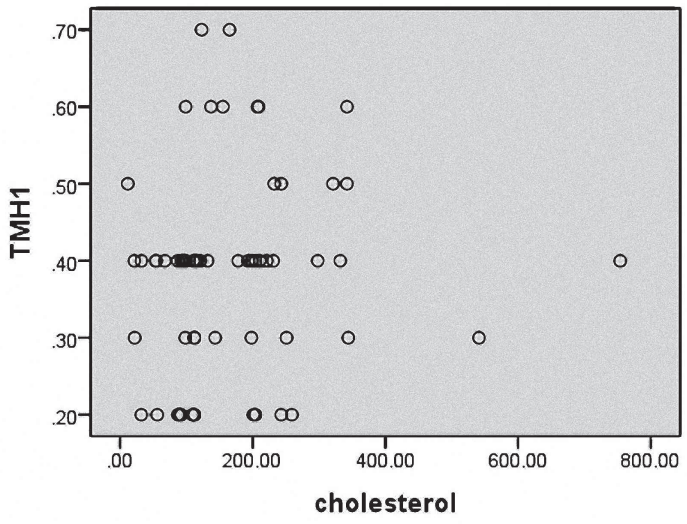

Figure 5.5—Scatter plot showing a weak positive correlation between cholesterol intake and TMH.

5.2 Logistic Regression

The data was transformed and a logistic regression was performed to see which of the different dietary factors best predict an OSDI score of less than 30, and therefore not suffering from dry eye syndrome. The model contained seven independent variables (PUFA encompassing EFA,

carbohydrates, vitamin C, vitamin E, cholesterol, calories and saturated fat). The full model containing these seven predictors was statistically significant X^2 (7, N = 200) = 71.42, p < 0.001. The strongest predictors of not suffering from dry eye were PUFA intake recording an odds ratio of 7.91. This indicated that subjects who had consumed PUFAs in their diet were over 7 times more likely to be not suffering from dry eye syndrome. Carbohydrate had an odds ratio of 8.01 and calorie intake had an odds ratio of 8.51 and therefore both were over 8 times more likely to predict not suffering from dry eye syndrome. When investigating relationships it is important to control for confounders, and potential confounders were identified to be smoking and contact lens wear. Therefore, the relationships between signs and symptoms of DES were re-analysed within subgroups of the cohort.

5.3 Non-smokers

The data was controlled for smokers. The sub-group of non-smokers was analysed and it was found that high OSDI scores were significantly associated with lower consumption of PUFA, showing a weak negative correlation (rho = – 0.24, p < 0.001),
carbohydrate (rho = – 0.21, p = 0.004), cholesterol (rho = – 0.13, p = 0.04),
calories (rho = – 0.33, p < 0.001) and saturated fat (rho = – 0.16, p = 0.04).
Greater TMH was associated with higher consumption of cholesterol showing a weak positive correlation (rho = 0.17, p = 0.02). Scatter plots 5.6 and 5.7 illustrate the stronger relationships.

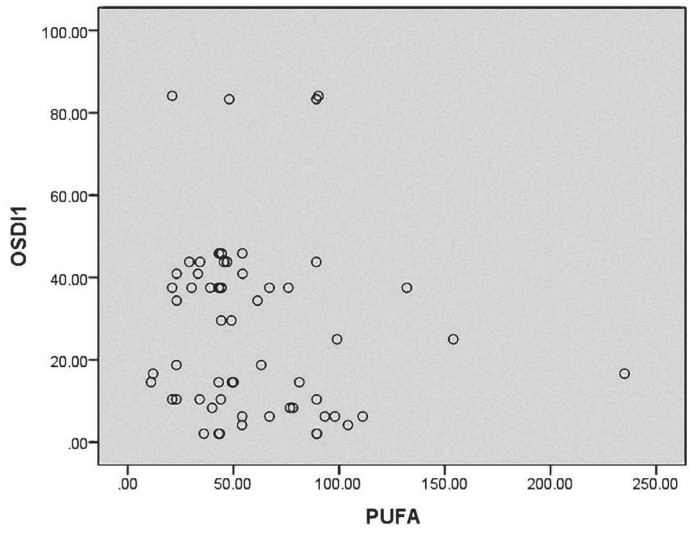

Figure 5.6—Scatter plot showing a weak negative correlation between PUFA intake and OSDI score in non-smokers.

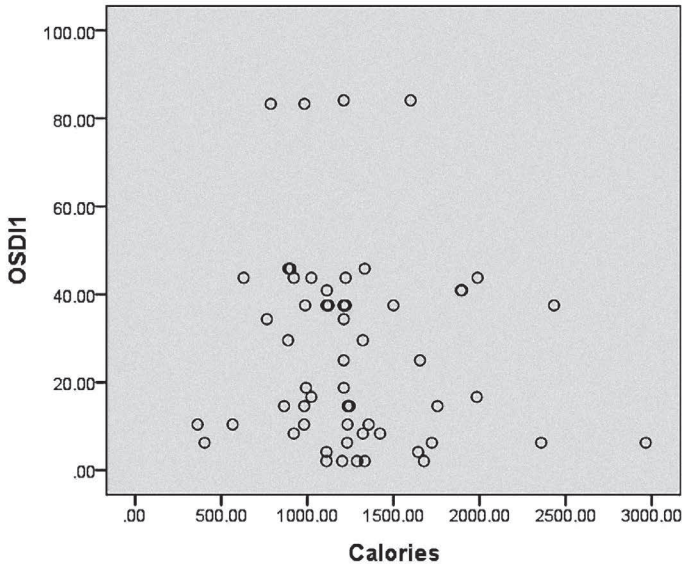

Figure 5.7—Scatter plot showing relationship between calorie intake and OSDI score in non-contact lens wearers

5.4 Non-contact lens wearers

The data was controlled for contact lens wear. The non-wearer sub-group was analysed and revealed that high OSDI scores were significantly associated with lower consumption of PUFA (rho = – 0.20, p = 0.01), carbohydrate (rho= – 0.25, p < 0.001), cholesterol (rho = – 0.15, p = 0.03), calories (rho= – 0.31, p < 0.001) and saturated fat (rho = – 0.16, p = 0.03), all showing a weak negative correlation. Greater TMH was associated with higher consumption of cholesterol (rho = 0.19, p = 0.01). See Figure 5.8, below, for the results.

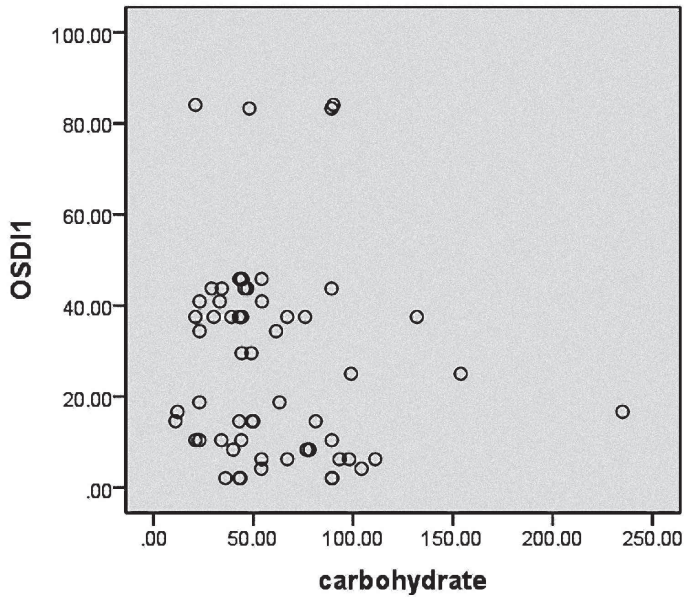

Figure 5.8—Scatter plot showing a weak negative correlation between carbohydrate intake and OSDI score in non-contact lens wearers

5.5 Bonferroni correction

A Bonferroni correction was used to reduce the chances of obtaining false-positive results. As there were thirty-five hypotheses being tested, the adjusted p value was calculated to be 0.001 (0.05/35). This correction was applied to the original results from the whole group and the two sub-groups.

Analysis on the whole group revealed that high OSDI scores were found to be significantly associated with lower consumption of PUFA (rho = – 0.21, $p < 0.001$), carbohydrate (rho = – 0.25, $p < 0.001$) and calories (rho = – 0.33, $p < 0.001$) even with this adjusted p value, all showing a weak negative correlation. See Table 5.1 for results.

Polyunsaturated fatty acids

	N	Mean / Standard Deviation	rho	p
OSDI score	200	24.00 / 20.01	– 0.21	<0.001

Carbohydrates

	N	Mean / Standard Deviation	rho	p
OSDI score	200	24.00 / 20.01	– 0.25	<0.001

Calories

	N	Mean / Standard Deviation	rho	p
OSDI score	200	24.00 / 20.01	– 0.33	<0.001

Table 5.1

N, rho and p values for all analyses on the whole group after applying a Bonferroni correction. Only significant relationships are shown.

5.5.1 Non-smokers

Analysis on the non-smokers revealed there to be a statistically significant relationships even with the adjusted p value. High OSDI scores were significantly associated with lower consumption of PUFA (rho = – 0.24, $p < 0.001$) and calories (rho = – 0.33, $p < 0.001$) with a weak negative correlation seen. See Table 5.2.

Polyunsaturated fatty acids

	N	Mean / Standard Deviation	rho	p
OSDI score	185	23.43 / 19.13	– 0.24	<0.001

Calories

	N	Mean / Standard Deviation	rho	p
OSDI score	185	23.43 / 19.13	– 0.33	<0.001

Table 5.2

N, rho and p values for the analyses for the non-smokers sub-group after applying a Bonferroni correction. Only significant relationships are shown.

5.5.2 Non-contact lens wearers

Analysis on the non-contact lens wearing sub-group revealed there to be a statistically significant relationships after the Bonferroni correction. High OSDI scores were significantly associated with lower consumption of carbohydrate (rho = – 0.25, p < 0.001) and calories (rho = – 0.31, p < 0.001), both showing a weak negative correlation. See Table 5.3, below.

Carbohydrates

	N	Mean / Standard Deviation	rho	p
OSDI score	183	24.51 / 19.96	– 0.25	<0.001

Calories

	N	Mean / Standard Deviation	rho	p
OSDI score	183	24.51 / 9.96	– 0.31	<0.001

Table 5.3

N, rho and p values for analyses for the non-contact lens sub-group after applying a Bonferroni correction. Only significant relationships are shown.

The limitations of this software include inability to extract omega-3 or omega-6 nutrient values from polyunsaturated fatty acids. To compensate for this, it was noted if the subject had recorded in their food diaries the consumption of at least one portion of oily fish or nuts.

5.5.3 Oily fish

The Mann-Whitney U Test was used to investigate the difference in dry-eye outcome measures between those subjects who had consumed at least one portion of oily fish and those who had not. The UK National Health Service advises that a single portion of oily fish may be defined as about 140g, after cooking.[132]

There was a significant difference in TMH: those who did consume oily fish had a significantly higher TMH ($Z = -2.23$, $p = 0.026$) than those who did not. There was also a counterintuitive higher mean OSDI score amongst those who consumed oily fish compared with those that did not ($Z = -2.079$, $p = 0.038$). The results are shown in Table 5.4, below.

	Consumed oily fish		Did not consume oily fish			
	N	**Mean / Standard Deviation**	**N**	**Mean / Standard Deviation**	**Z**	**p**
OSDI score	88	26.34 / 18.98	112	22.10 / 0.71	−2.079	0.038
TMH (mm)	88	0.41 / 0.12	112	0.37 / 0.14	−2.23	0.026
NITBUT (seconds)	88	4.24 / 1.95	112	3.95 / 1.64	−0.016	0.987
TBUT (seconds)	88	4.06 / 2.05	112	3.35 / 1.28	−1.168	0.243
Total corneal staining	88	2.00 / 2.54	112	2.57 / 4.36	−0.063	0.950
Total conjunctival staining	88	10.48 / 4.26	112	9.70 / 4.80	−1.598	0.110

Table 5.4

Z and p values for relationships between dry eye outcomes and consumption of at least one portion of oily fish.

5.5.4 Nuts

The Mann-Whitney U Test was used to investigate the difference in dry-eye outcome measures between those subjects who had consumed at least one portion of nuts and those who had not. The UK National Health Service advises that a single portion of nuts may be defined as approximately one ounce, or 28g, which is about as much as will fit in the palm of a hand.[132]

The results showed that those consuming nuts had a significantly lower OSDI score ($Z = -4.018$, $p < 0.001$), higher TMH ($Z = -2.867$, $p = 0.004$), and longer NITBUT ($Z = -3.616$, $p < 0.001$) and TBUT ($Z = -4.174$, $p < 0.001$) than those that did not. The results are shown in Table 5.5, below.

	Consumed nuts		Did not consume nuts			
	N	Mean / Standard Deviation	N	Mean / Standard Deviation	Z	p
OSDI score	114	18.69 / 15.42	86	31.40 / 23.17	– 4.018	<0.001
TMH (mm)	114	0.41 / 0.13	86	0.35 / 0.13	– 2.867	0.004
NITBUT (seconds)	114	4.42 / 1.75	86	3.65 / .75	– 3.616	<0.001
TBUT (seconds)	114	4.13 / 1.81	86	3.10 / 1.40	– 4.174	<0.001
Total corneal staining	114	1.93 / 2.77	86	2.84 / 4.57	– 1.420	0.156
Total conjunctival staining	114	9.72 / 4.29	86	10.47 / 4.92	– 1.397	0.162

Table 5.5

Z and p values for relationships between dry eye outcomes and consumption of at least one portion of nuts.

5.6 Summary

Statistical analysis on the whole group concluded that high OSDI scores were significantly associated with lower consumption of PUFAs, carbohydrate, cholesterol, calories and saturated fat. After the Bonferroni correction was applied with the adjusted p value, analysis confirmed higher OSDI scores were significantly associated with lower consumption of PUFAs, carbohydrate and calories.

Both the non-smokers and non contact lens wearing sub-groups revealed high OSDI scores significantly associated with lower consumption of PUFAs carbohydrate, cholesterol, calories and saturated fat. Further analysis after the Bonferroni correction confirmed that high OSDI scores were significantly associated with lower consumption of PUFAs and calories in the non-smokers

sub-group and with lower consumption of carbohydrates and calories in the non contact lens wearing sub-group.

Greater TMH was seen in subjects who had higher consumption of cholesterol. This applied also when controlling for smoking and contact lens wear. There was also a significant difference in TMH in those who consumed nuts and oily fish as had a significantly higher TMH than those who did not. Also, results showed that those consuming nuts had a significantly lower OSDI score, together with increased NITBUT and TBUT, than those who did not.

There was also a counterintuitive higher mean OSDI score among those who consumed oily fish compared with those who did not.

Chapter 6: Intra-practitioner reliability of outcome measures

Intra-practitioner reliability of the outcome measures was explored: this entailed inviting 58 participants to return to the practice to be reassessed by the researcher using the same techniques as before (described in Chapter 2).

6.1 Results from repeated assessments

According to the Kolmogorov-Smirnov statistic, all variables from the original 200 subjects and the repeated 58 were not found to be normally distributed (significance value less than 0.05).

After measurements had been repeated on the 58 subjects, it was important to assess for each of them the level of agreement between the initial and second sets of measurements—any discrepancies needed to be evaluated to see how significant they were. Therefore, the differences between the clinical outcome measurements from the two visits were related to the mean and a log transform was performed. The mean and standard deviation of the differences were calculated. The mean difference should be zero since the same method of measurement was used. If the mean difference is significantly different from zero, it will not be possible to use the data further because one or both of the two sets of measurements might not reflect the true situation—due to measurement errors, changes in the subject's circumstances, and so on. The definition of a repeatability coefficient adopted by the British Standards Institution requires that 95% of differences should be less than two standard deviations.[128]

It was determined whether there was a difference between each set of two measurements and how they varied from one another. A one-sample t-test was conducted with the null hypothesis that there is zero difference between the two measurements and that they completely agree. The t-test on the results from the OSDI score outcome measures revealed significance value of less than 0.05 between the two visits (mean difference = – 9.48879 and p-value was 0.000). Hence, this was regarded as significantly different from one visit to the other. This result did not show a useful level of agreement and therefore Bland-Altman analysis was not conducted. All other clinical outcome measures revealed a significance value of greater than 0.05, confirming that the measurements from the two separate visits were not significantly different from one another (p-value >0.05). For these clinical measures, a Bland-Altman analysis was used to assess the level of agreement of the outcome measures between the two visits. The mean difference between each set of two measurements was determined to see if they differed significantly from zero and therefore to check if there was a resultant bias. Linear regression was carried out to confirm or reject the null hypothesis that the coefficient for mean is zero.

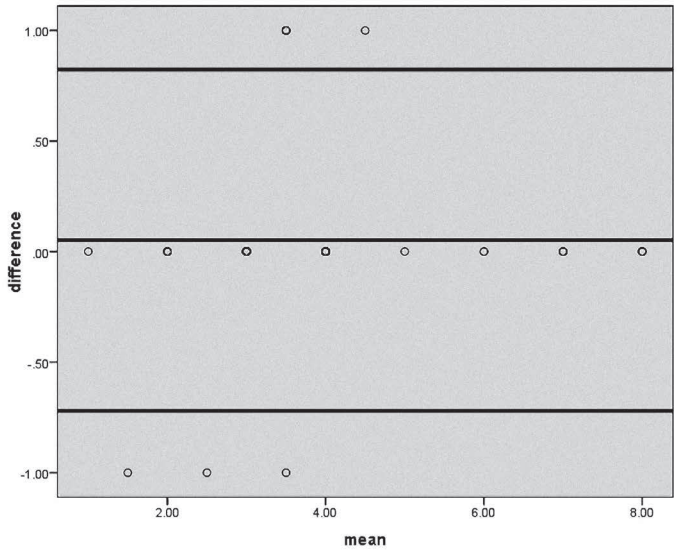

Fig 6.1—Bland-Altman plot, NITBUT.

The Bland-Altman analysis on NITBUT clinical measure on the two occasions indicated a mean difference of 0.0517 with 95% limits of agreement between the two visits and measurements ranged from – 0.0519 to 0.1553. Linear regression was carried out to confirm or reject the null hypothesis that the coefficient for mean is zero. In this instance, the beta value was 0.0171 and did not reveal a statistically significant result as p = 0.585: therefore it was concluded that there was no proportional bias and confirmed that there was no trend showing more data points above or below the mean score. See Figure 6.1, above.

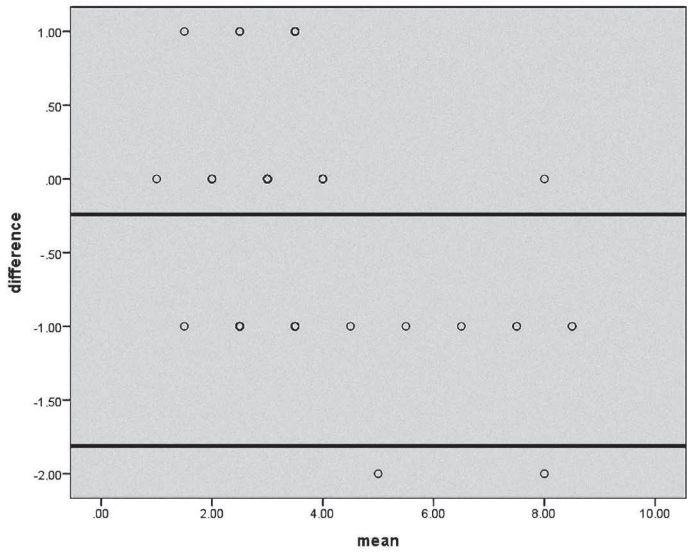

Figure 6.2—Bland-Altman plot, TBUT.

The Bland-Altman analysis on TBUT clinical measure on the two occasions indicated a mean difference of – 0.2414 with 95% limits of agreement between the two visits and measurements ranged from – 0.4521 to 0.0306. Linear regression was carried out to confirm or reject the null hypothesis that the coefficient for mean is zero. In this instance, beta value was – 0.171 and revealed a statistically significant result as p = 0.004: therefore there was a proportional bias as seen in Figure 6.2 with a cluster of data points below the mean score. See Figure 6.2, above.

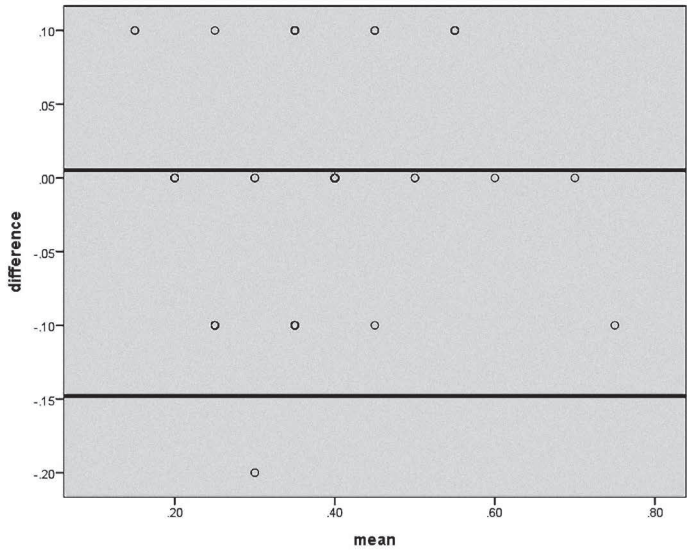

Figure 6.3—Bland-Altman plot, TMH.

The Bland-Altman analysis on TMH clinical measures on the two occasions indicated a mean difference of 0.0052 with 95% limits of agreement between the two visits and measurements ranged from – 0.0154 to 0.0257. Linear regression was carried out to confirm or reject the null hypothesis that the coefficient for mean is zero. In this instance beta value was 0.095 and did not reveal a statistically significant result as p = 0.266: therefore there was no proportional bias and that there was no trend showing more data points above or below the mean score.
See Figure 6.3, above.

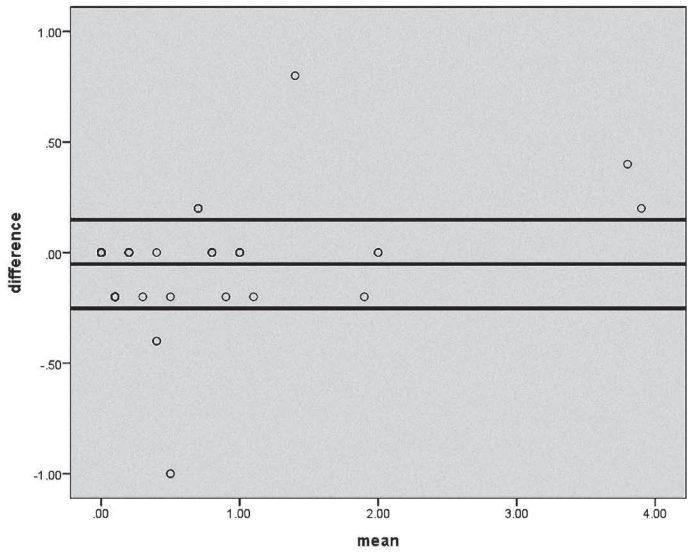

Fig 6.4—Bland-Altman plot, corneal staining.

The Bland-Altman analysis on corneal staining indicated that there is mean difference of – 0.0517 with 95% limits of agreement between the two visits and measurements ranged from – 0.1175 to 0.0140. Linear regression was carried out to confirm or reject the null hypothesis that the coefficient for mean is zero. In this instance beta value was 0.082 and did not reveal a statistically significant result as p = 0.053: it was concluded that there was no proportional bias and that there was no trend showing more data points above or below the mean score.
See Figure 6.4, above.

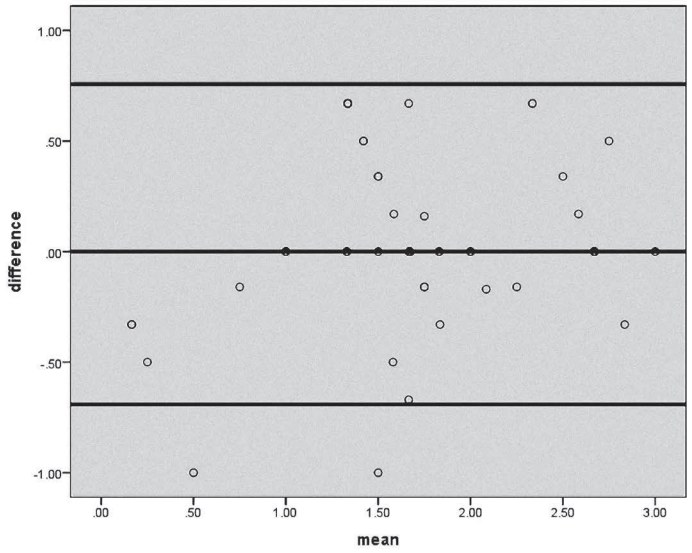

Fig 6.5—Bland-Altman plot, conjunctival staining.

The Bland-Altman analysis on conjunctival staining indicated that there is mean difference of 0.0329 with 95% limits of agreement between the two visits and measurements ranged from – 0.0642 to 0.1300. Linear regression was carried out to confirm or reject the null hypothesis that the coefficient for mean is zero. In this instance, beta value was 0.134 and did not reveal a statistically significant result as p = 0.079 therefore it was concluded there was no proportional bias and that there was no trend showing more data points above or below the mean score. See Figure 6.5, above.

6.2 Summary

The Bland-Altman analysis on clinical measurements of TBUT, NIBUT, TMH and corneal and conjunctival staining on the two occasions showed there was no proportional bias and confirmed there was no trend showing more data points above or below the mean score and therefore there was consistency in intra-practitioner repeatability. These clinical measurements can vary and consistency of interpretation is key as other studies have found that clinical outcome measurements were highly variable under all conditions[130,131]

The t-test on the results from the OSDI score outcome measures revealed significance value of less than 0.05 between the two visits. Hence, this was regarded as significantly different from one visit to the other. This did not show a useful level of agreement and is an example of patient-reported symptoms which can be only moderately repeatable from visit to visit, and thus confirmed that procedures clinically used to diagnose and monitor dry eye syndromes can in this case be regarded as unrepeatable.[129]

Chapter 7: Discussions and Conclusions

Every eye care professional and optometrist will encounter patients who present with the overt symptoms of dry eyes—such patients complain of 'soreness', 'dryness', 'grittiness' and other sources of discomfort. However, the degradation of ocular optical qualities resulting from DES often results in patients presenting to their optometrist with visual impairments they have noticed during critical tasks such as driving or viewing a computer screen, phone, or tablet. These impairments result from changes in aberration caused by changes in tear film.[72]

Symptoms of dry eye can be treated with moderate success, but long term solutions are needed both to prevent its occurrence and to ameliorate the symptoms more effectively when it does occur.

The aim of the present study was to investigate the relationship between diet and clinical assessment of the tear film in routine optometric practice and add to existing studies. Dry eye syndrome is widespread in the population, and, although not sight-threatening, it can reduce quality of life. For this reason alone, research into DES is well worthwhile, and one approach has been to investigate the possible relationship between diet and DES. A number of studies have been conducted in various parts of the world but few studies and little data are available for the UK.[10,13,15,16,17,18,20,29,43,44,45,59,50,52,64,80,89,90,91,94,95,96,101,112,113, 115,116]

The *a la calc* nutritional software used in this study is new, having been established just under five years ago. It has its limitations, but the alternatives had greater limitations and showed no improvements. The inability of *a la calc* to separate omega-3 and omega-6 EFA's was a major disadvantage and essentially a flaw: this study attempted to overcome that aspect by having the consumption of nuts and oily fish recorded in patients' food diaries. It would have been valuable to assess blood serum levels of nutrients but this was not possible due to the optometric practice-based nature of the research.

There are a number of techniques that can be used for tear film assessment in routine optometric practice, but it is it is impractical to conduct every test on every patient. The clinical outcome measures used in this study were chosen as they were readily available in optometric practice and representative of the tests readily conducted by optometrists.

The tear film is inherently variable during the day and unfortunately this remained a flaw for this study as tear film evaluations were carried out on various days and times of day. All the subjects were recruited from only one practice and this resulted in another major limitation. Also, the average age of the subjects recruited in this study was 56 years with larger proportions of females and non smokers than males and smokers. Furthermore, these demographics were not representative of the UK as all the subjects were Caucasian and comparisons could not be made between different ethnicities. To overcome this deficiency, further research would need to be conducted in various locations more representative of the UK population as regards ethnicity, age and lifestyle habits. This is especially important as it has been confirmed that DES can be significantly associated with environment and ethnicity,[138,148,149] For example, in Japan, prevalence of DES has been recorded as high as 33%.[136]

The present study consisted of 200 participants: the mean OSDI score was 24 (median 17), which is well below the cut-off for diagnosis of DES. The percentage of participants with an OSDI score of over 30 was 43%. When comparing the subgroups, the only significant finding revealed that females had a significantly higher OSDI score than males (26 compared with 22). Females also revealed dry eye outcomes such as lower TBUT, NITBUT and TMH compared with males. This is also consistent with previous research sources[1,10,13,14,16,17,134] and in more recent studies conducted in the United States with two large cross-sectional surveys, the Women's Health Study and the Physician's Health Studies, demonstrating that the prevalence of dry eye or severe dry eye symptoms was 7.8% in women and 4.3% in men aged 50 years and older. Sixteen larger studies have reconfirmed a link between being female and having a higher risk of DES.[10,14] Within the 25,665 postmenopausal women in the Women's Health Study, a significant association was made with an increased risk of clinically diagnosed dry eye syndrome and severe symptoms.[16]

Increasing age and the prevalence of dry eye syndrome is a well known association both in males and females.[1,10,16,17,94] However, in this study, there was no significant difference between the sub-categories for age when comparing those participants who were aged 50 years and under with the sub-group over the age of 50 years. This counter-intuitive result could be explained by the fact that the average ages for the two categories were very similar (the result was reflective of the patient base and general catchment of the research location). A more diverse age range would be required to fully understand the overall relationship between increasing age and DES.

Statistical analysis on the whole group concluded that high OSDI scores were significantly associated with lower consumption of polyunsaturated fatty acids and there was a weak negative correlation with the intake of carbohydrate, cholesterol, calories and saturated fat. This is consistent with studies mentioned earlier.[25,27,32, 33,34,35] After controlling for contact lens wear and smoking and after applying the Bonferroni correction with the adjusted p value, analysis confirmed higher OSDI scores were significantly associated with lower consumption of PUFAs. This is consistent with findings by other researchers,[139,140] However, the inverse relationship seen between OSDI score and the intake of carbohydrate and calories is a new finding and one which will merit further investigation. Greater TMH was seen in participants who had higher consumption of cholesterol. This applied also when controlling for smoking and contact lens wear. There was also a significant difference in TMH in participants who consumed nuts and oily fish as these persons had a significantly higher TMH than those who did not. Also, results showed that those consuming nuts had a significantly lower OSDI score, together with increased NITBUT and TBUT, than those who did not.

Objectively correlating changes in dry eye syndrome with blood levels of omega-3 EFAs has not been done in a large-scale multisite study. There is also no consensus on the dose, composition, length of treatment, and so on, with omega-3 or omega-6 EFAs. Increased quality evidence on the usefulness of over-the-counter supplements is needed to enable doctors and eye care practitioners to confidently outline specific treatment recommendations for using omega-3 EFAs in DES.[98] Indeed, a comparative study in India demonstrated the beneficial effect of orally administered omega-3 EFAs in alleviating dry eye symptoms, decreasing tear evaporation rate

and improving symptoms in patients suffering dry eye related to computer vision syndrome.[99] The use of EFAs in dry eye needs to be further investigated, particularly as EFAs have already shown their usefulness in systemic conditions.

The key to understanding the potential benefits of EFAs is to gain a clearer understanding of their physiological interactions and to ensure that appropriate amounts of each EFAs are consumed. Individuals with dry eye often seek advice from doctors and eye care practitioners such as optometrists: these professionals should familiarise themselves with EFAs and their benefits and recommend to patients that they increase their consumption of omega-3 EFAs to help alleviate the symptoms. Increasing consumption of oily fish helps to reduce DES symptoms and full spectrum multi-vitamin/mineral/EFA nutritional supplements provide the opportunity to optimise dietary defences against DES.[150] Future studies in patient-reported outcome measures for dry eye and ocular surface disease should address these issues.

Although this study revealed no statistically significant difference in dry eye outcome measures between smokers and non-smokers, it has also been recorded that various lifestyle habits such as smoking can cause adverse effects on the precorneal tear film with a strong association between smoking and tear film instability.[133,134,135] As mentioned earlier, it is well known that smoking can cause adverse effects on the tear film as seen in much larger studies where more of participants examined had significant smoking habits.[134,135] Future directions for this study should explore the current effect of shisha and E-cigarettes. Although relatively scarce, the existing data with regards to DES and socioeconomic background and lifestyle habits does suggest a substantial link.[140,141,142,143,144] Future aspects of study should include other factors such as mental health and general well-being and fitness. This has been briefly explored with research into the association of DES with anxiety and depression which has shown a positive association and would be of clinical relevance.[145,146,147]

This study was carried out on a population of 200 subjects drawn from patients of an optometric practice located in the east of the United Kingdom, resulting on a cohort not representative of the general population of the UK. Nevertheless, the results have suggested further areas for research including investigation of the inverse relationship found between OSDI score and the intake of PUFAs, carbohydrate and calories.

The results and information from the present thesis reinforce suggestions that optometrists and medical professionals such as family doctors would be well placed not only to treat the symptoms of DES by prescribing lubricating eye drops or tear substitutes but should also be more aware of the effects of dietary intake on DES and the possibility of ameliorating symptoms by suggesting changes to the patient's diet. No doubt further research by others will clarify how best to use changes in diet in the treatment of DES.

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APPENDICES

Appendix 1
Ethical approval

Aston University
Birmingham

Aston Triangle
Birmingham B4 7ET
United Kingdom
Tel +44 (0)121 204 3000

www.aston.ac.uk

Memo

Life and Health Sciences Research Ethics Committee's Decision Letter

To : *Dr Hannah Bartlett*

Cc: Rachel Moorhouse, administrator to the Life and Health Sciences Research Ethics Committee

From: Dr Doina Gherghel

Chair of the Life and Health Sciences Research Ethics Committee

Date: 11/07/2012

Subject: Project **: # 367. The relationship between dietary intake and dry eye.**

The documentation and additional information for the above proposal has been considered by the Chair of the LHS Research Ethics Committee.
Please see below for details of the decision and the approved documents:

Reviewer's recommendation: Approved.

Reviewer's comments: This study may now proceed.

Please see the tabled list below of approved documents:

Documentation	**Version/s**	**Approved**
Consent Form	1	✓
Participant Information Sheet (PIS)	1	✓
Protocol	1	✓
Risk Assessment	N/A	
Questionnaires	1	✓
Other (please detail):		

After starting your research **please notify the LHS Research Ethics Committee of any of the following at; lhs_ethics@aston.ac.uk**

Please quote the original project reference number with all further correspondence.

- **Substantial amendments** – Any amendment should be sent as a Word document, with the amendment highlighted.
  Please include a version number and amended date to the file name of any amended documentation (e.g. Ethics Application #100 Protocol v2 amended 17/02/12) - When any amendments are submitted they need to be accompanied by new protocols / PIS etc. as necessary, with the new version numbers included.
- **New Investigators**
- **The End of the Study**

All necessary documents are available to download from; **http://www.ethics.aston.ac.uk/documents-all**

Please note that these documents can ONLY be opened using **Mozilla Firefox or the latest Internet Explorer version (IE9).**

Statement of Compliance
The Committee is constituted in accordance with the Government Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Yours sincerely

Aston University

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Dr Doina Gherghel
Chair of the LHS Research Ethics Committee

The study has been approved by the Aston University Human Sciences Ethical Committee on 11th July 2012 – ethics application number 367.

Because of ethical issues raised by the design of this protocol the inconvenience in attending and carrying out any additional tests, recording of data relating to results obtained from participants and risks associated with questionnaires, it is necessary to put the following additional protections in place for the research participants:

Anonymity

This will be preserved by the removal of identifiers and the use of ID numbers or pseudonyms, breaking the link between data and identifiable individuals.

Risks and benefits

There is no known physical or psychological risk. Patients may gain benefits from this study in relation to dry eye and their diet.

Informed consent

Verbal and signed written informed consent will be obtained for participation.

Voluntary Participation

Participation will be voluntary; participants will not be coerced into participating by offering incentives to do so. Participants may withdraw from taking part at any point.

Privacy and confidentiality

Responses to questionnaires and tear film analysis will be confidential. Presentation of the results will omit any factor which personally identifies the participant, therefore, maintaining confidentiality. All data and questionnaires will be kept in a locked drawer unless being processed. When input on to a computer the information will be stored on a password-protected PC. The data will be analysed in a private study area by the chief investigator.

By instituting these additional protections, any risks have been appropriately minimised, and a reasonable and ethically acceptable balance between risks and benefits has been established.

Appendix 2
Subject Information sheet and consent form

Research workers, school and subject area responsible

Optometrist Researcher:

Sheeraz Janjua BSc(HONS) DipSv MCOptom MIoD

Project Title

The relationship between diet and dry eye syndrome

Invitation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?

Dry eye is a common condition, increasing with age. Current treatments deal with the management of the tear film. Relief may be achieved through instillation of artificial tears, although this does not last long because these topical treatments generally treat the symptoms and are unable to resolve the underlying cause. Large prevalence studies and studies relating dietary intake with dry eye have been conducted in other parts of the world; however, little data is available for the UK. There is evidence to suggest that certain dietary constituents, such as essential fatty acids (EFA), particularly omega-3 EFA, may be effective in dealing with the underlying causes and reduce the risk for dry eye. This relationship can be explored in detail using food diaries, dry eye questionnaires and tear analysis.

What will happen to me if I take part?

This research will involve patients from research centres and optometry clinics based in the UK. Participation will involve a single visit to one of the centres or clinics.

You will be asked to:

- Answer a 24 hour food recall questionnaire
- Complete a health and lifestyle questionnaire
- Complete a dry eye questionnaire
- Consent to having your tear layer assessed by the researcher using standard optometric techniques

Are there any potential risks in talking part in the study?

There is no known psychological risk. Physical risks include a minimal, brief, discoloration of the eye and the possibility of a corneal abrasion. This risk will be minimised by giving you accurate instructions when the researcher performs the tests.

All data will remain anonymous at all times. Data analysis will be carried out by clinicians and statisticians. Any other members of the research team will only be given access to the database after your identity has been removed.

Do I have to take part?

No, you do not have to participate if you do not wish to do so. You are free to withdraw at any time from the project without any penalty.

Expenses and payments:

There are no expenses or payments for participation in this project.

Will my taking part in this study be kept confidential?

Yes, your participation in the study will be fully confidential. There will be no way to link any research data to any individual participant. All data will be anonymised.

What will happen to the results of the research study?

We aim to publish the results of this project. However, there will be no direct reference to any individual's data in any publication.

Who is organising and funding the research?

The researcher is organising the study. There is no funding for this research project.

Who has reviewed the study?

The research has been submitted for approval by Aston University's Life and Health Sciences Research Ethics Committee.

Who do I contact if something goes wrong or I need further information?

Please feel free to contact the chief researcher: Mr Sheeraz Janjua (janjuasa@aston.ac.uk)

Who do I contact if I wish to make a complaint about the way in which the research is conducted?

If you have any concerns about the way in which the study has been conducted, then you should contact Aston University's Life and Health Sciences Research Ethics Committee on j.g.walter@aston.ac.uk or telephone 0121 204 4665.

Study Number:

Patient Identification Number:

VOLUNTEER CONSENT FORM

Title of Project: The relationship between dietary intake and dry eye.

Name of Chief Researcher: Mr. Sheeraz Janjua BSc (Hons) DipSv MCOptom MIoD

Please tick box

1. I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Name of Person taking consent (If different from Researcher)	Date	Signature
Researcher	Date	Signature

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

Appendix 3
Advertisement for recruitment of subjects

Scunthorpe Telegraph

People needed for eye research

There is increasing evidence to suggest that certain dietary constituents, such as essential fatty acids, reduce the risk of Dry Eye Syndrome.

This relationship will be explored using food diaries, dry eye questionnaires and tear analysis by Optometrist at O'Briens Opticians in Brigg – Sheeraz Janjua (pictured).

Mr Janjua is currently studying for a Doctor of Optometry Postgraduate qualification at Aston University and is seeking to assess people who suffer from Dry Eye Syndrome as part of his research thesis.

People interested in participating should contact the practice on 01652 653595.

Appendix 4
INDES Health and lifestyle questionnaire

INDES
International Nutrition and Dry Eye Study

Study Number:

Unique ID (**Patient Identification Number)**

1. _____

2. Male/Female _____

3. Date of Birth _____

4. What is your ethnic background? (circle those which apply)

    - Asian or Asian British – Bangladeshi/Indian/Pakistani
    - Black or black British – African/Caribbean
    - Chinese
    - Mixed – white and Asian/White and black African/ White and black Caribbean
    - Other Asian (please state) _____
    - Other Black (please state) _____
    - Other mixed (please state) _____
    - White – British/Irish
    - Other white (please state)_____
    - Other ethnic group (please state) _____

5. Please list any general health problems you have (e.g. blood pressure or diabetes)

    _____

    _____

    _____

6. Do you take any tablets or medicines? If so, please list the names below _____

    _____

    _____

    _____

7. Do you take any nutritional supplements (e.g. vitamins)? If so, please provide the name or brand _____
_____
___

8. Do you smoke? _____

9. If so, how many cigarettes per day_____

10. If you don't smoke at the moment but you have done previously, please give deta

11. ils_____
_____

11. Please give information about any previous visits to an eye clinic or eye specialist

_____
_____
_____

12. Are you a contact lens wearer?_____

a. If so, what type of lenses do you wear?_____
_____
_____

b. How many days per week do you wear your lenses?_____

c. How many hours per day do you wear your lenses?_____

THANK YOU FOR YOUR TIME.

Sheeraz Janjua

janjuasa@aston.ac.uk

Appendix 5
24-hour Food Recall Questionnaire

Food to be described as accurately as possible using visual props:

For example:

One small or large bowl of Wheatabix with skimmed milk

Two slices of toast thinly or thickly spread with butter

Brown, White or Wholemeal bread

Skimmed or semi-skimmed milk

Large, medium or small apple

Accurate estimates of the food and drink consumed using visual props:

For example:

One small cup of coffee or one large mug of tea

One or two biscuits

One packet of crisps

Please ask the participant to recall all the food and drinks they consumed yesterday from waking to sleeping. If yesterday was a weekday, please also ask the participant to recall all the foods and drinks consumed during one preceding weekend day. If the previous day was a Sunday, please ask the participant to recall all the food and drinks they consumed the preceding Friday.

Remember to include all foods and drinks consumed at home and at other places such as at work and in restaurants.

Study Number

Patient Identification Number

24 Hour Food Recall Questionnaire: weekday	
Breakfast:	**Evening meal:**
Lunch:	**In between snacks:**

Study Number:

Patient Identification Number

24 Hour Food Recall Questionnaire: weekend day (please specify)	
Breakfast:	**Evening meal:**
Lunch:	**In between snacks:**

THANK YOU FOR YOUR TIME.

Sheeraz Janjua

janjuasa@aston.ac.uk

Appendix 6
OSDI Questionnaire


Aston University
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Appendix 7
Clinical data collection form

Please do not evaluate the tear film until 10 minutes after the subject has arrived in order to allow the effect of external conditions on the tear film to settle

- Data can either be entered directly into the Bristol Online Survey (see link on INDES website) or into the INDES spreadsheet (which can be downloaded from the INDES website).

Date:_____ **Study location:**_____

Subject unique identification code:_____

(Created by using three letters of the mother's maiden name followed by the last three digits in the phone number)

Chosen eye: R ☐ **L** ☐ (Right eye unless affected by pathology other than dry eye)

For all participants:

Vision in chosen eye using a standard LogMAR chart (standard viewing conditions)

Vision: _____

For distance spectacle/contact lens wearers:

Visual acuity in chosen eye using a standard LogMAR chart (standard viewing conditions)

VA: _____

Non-invasive tests:

Manual Keratometry (three readings, plus an average, allowing one minute between readings).

1st_____ 2nd_____ 3rd_____ Ave_____

Central Tear Meniscus Height:_____(mm)

Invasive tests:

Fluorescein tear break up time (FTBUT):_____(seconds).

Ocular surface fluorescein staining assessment using the Efron grading scale (http://eprints.qut.edu.au/11857/**):**

Cornea: _____ Bulbar conjunctiva: _____