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Meibomian gland dysfunction and Dry Eye Symptoms of Fitted and Over the Counter Contact Lens Wearers compared with non-Contact Lens Wearing Controls

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Contents

Abstract

Meibomian gland dysfunction (MGD) is a chronic and diffuse abnormality of the meibomian glands (MGs) that occurs in 38% of contact lens (CL) wearers, though the influence of CLs on MGD is equivocal. A topical literature review examining the influence of CL wear on MGs based on 22 research studies found 15 studies reporting an effect of CLs on MGs vs. seven studies who did not. Discrepencies may be due to varying definitions of MGD, the type of questionnaire or assessment method, CL type, and measurement of one vs. both eyelids. The review concluded that the effect of CL wear on MGD should be addressed with a prospective study that includes a non-CL wearing controls, and employing an objective assessment of MGs.

The Cobra HD meibographer is an objective instrument used in research studies whose reliability has not been investigated. Therefore, its inter-session repeatability (ISR), inter-examiner reproducibility (IER) and within-subject variability (WSV) was evaluated in participants with and without dry eye (DE) symptoms based on their Ocular Surface Disease Index (OSDI) questionnaire. Seventy-two participants (mean age: 23±5 years, 36 asymptomatic) were examined by Examiner 1 on two different sessions (S1, S2) to calculate the ISR, and seventy-four (mean age: 23±5 years, 37 asymptomatic) were measured on the same day by two examiners (E1, E2) to determine the IER. WSV was determined from three consecutive measurements of the same eyelid captured by each examiner. Mean MG loss of the upper (S1:13.5±9.5%,S2:12.8±8.5%, E1:12.7±8.2%, E2:13.1±8%) and lower eyelids (S1:7.5±6.9%,S2:7.3±6.3%, E1:7±6.2%, E2:7.4±6.2%) was not significantly different between sessions or examiners for all cohorts for both eyelids. The ISR within-subject standard deviations (Sw) for the upper and lower eyelids were 1.3% and 1.0%; mean difference (md) was 0.7±3.5% [CI:-6.25%- 7.62%] and 0.1±2.1% [CI:-3.94%- 4.17%], respectively. The IER ICC values were >0.86 for all conditions, Sw was 1.3% and 1.2% with md of -0.4±3.2% [CI:-6.65%- 5.9%] and -0.4±2.9% [CI:-6.15%- 5.31%], respectively. The WSV Sw values were <1.4%, and ICC values were >0.89 for both eyelids, examiners and sessions. Thus, the Cobra meibographer is repeatable, reproducible and has low WSV.

In Israel CLs may be purchased over-the-counter (OTC) without a prescription or follow-up. Therefore, the relationship between MGD and CL wear was assessed in CL wearers who were followed-up (FLU) and those who were not followed-up (non-FLU), and non-CL wearing controls. Habitual LogMAR visual acuity (VA), Meibum Quality Score (MQS), Meibum Expressibility Score (MES), MG loss, lid margin abnormalities and DE symptoms of the right eyes of the cohorts were compared using Kruskal-Wallis for ordinal and Pearson Chi-Square test for categorical variables, respectively. Univariate logistic regression examined if CLs are an independent risk factor for MG abnormalities. Of the 128 participants (74% female); 31 were FLU, 43 were non-FLU and 54 were controls (mean ages: 22.2±3.1, 22.3±3.5, and 23±4.6, respectively). non-FLU CL wearers had lower VA than controls (0.82±0.17 vs. 0.93±0.12, p=0.002) and more DE symptoms (3.7±2.4 vs. 2.3±2.1, p=0.002). CL wearers (FLU:0.6±0.7, p<0.01, non-FLU :0.8±0.9, p<0.0008) had worse MES than controls (0.2±0.5) and more MG loss (FLU:16.9%±8.8%, p=0.02, non-FLU:18.6%±11.3%, p=0.001) than controls (11.2%±6.8%). CL wear was associated with corneal staining (odds ratio(OR)=3.42, 95% confidence interval(CI):1.16–10.11, p=0.03 and OR=5.23, 95%CI: 1.89–14.48, p=0.001, respectively) and MG loss (OR=10.47, 95%CI:1.14–96.29, p=0.04 and OR=16.63, 95%CI:1.96–140.86, p=0.01, respectively). non-FLU CL wear was also associated with abnormal MQS (OR=12.87, 95%CI:1.12–148.41, p=0.04), conjunctival staining (OR=12.18, 95%CI:3.66–40.51, p=0.0005), and lid margin telangiectasia (OR=3.78, 95%CI:1.55–9.21, p=0.003). CL wearers had significantly more morphological and functional abnormalities than controls. non-FLU resulted in more DE symptoms, poorer VA, and were an independent predictor for more functional and morphological abnormalities than FLU CL wearers, emphasizing the importance of proper fitting and aftercare.

Key words: Meibomian gland dysfunction, dry eye, contact lens, over-the-counter, follow-up, after-care.

2. Introduction

Meibomian gland dysfunction (MGD) has been defined by the subcommittee of the International Workshop on MGD as a chronic and diffuse abnormality of the meibomian glands (MGs) that is characterized by a terminal duct obstruction and/or qualitative or quantitative changes in gland secretion. [1] MGD can affect the tear film, and induce symptoms of eye irritation, inflammation, and ocular surface disease, [1] and is a leading cause of evaporative dry eye (DE). [2] The prevalence of MGD is as high as 70%, [3] and it is a significant public health problem. [4]

MGD diagnosis includes an assessment of symptoms, gland function and morphology. Symptoms are assessed using questionnaires. [3] Function is assessed by the meibum quality and expressibility while morphology includes an evaluation of the MGs and eyelid margin abnormalities. [5] Gland morphology can be evaluated using meibography, which allows observation [6] and in vivo evaluation of the MG structure. [7]

This doctoral thesis describes the results of three sub-studies. The first, is a topical review examining research studies that examined the effect of CL wear on the morphology and functional of the MGs. One conclusion of the review was that there is a need for a reliable objective device, in determining the existence of MGD. Therefore, the second study examined the repeatability and reproducibility of the Cobra HD meibographer.

The Cobra HD (csoitalia.it) non-mydriatic digital fundus camera [7] with Phoenix semi-automated software that is specially designed for MG analysis, [8] employs a ‘vectorised’ tool with Beziers curves that adapts rapidly to the eyelid shape. [9] The Phoenix software requires that the examiner mark the area of the eyelids and the location where the glands are observed, while the software subsequently calculates the percentage of MG loss. [10] Though considered an objective measure, the measurement may vary due to the variation in the examiners’ identification of the borders of the tarsus and MGs, leading to inter-observer variability. [6], [11]

Despite the widespread use of the Cobra HD meibographer in research studies, the within-subject variability (WSV), inter-session repeatability (ISR)

and inter-examiner reproducibility (IER) of this device have not been examined. These outcomes are important when determining the utility of instruments used in comparative and observational studies, and clinical measurements used to diagnose diseases such as MGD. [4] Therefore, the second study investigated the WSV, ISR and IER of the Cobra HD fundus camera meibographer in participants with and without dry eye symptoms.

MGD has been estimated to occur in 38% of CL wearers, [3] however the influence of CLs on MGD is equivocal. [12]–[14] On the one hand, CL wear has been shown to induce morphological and functional changes in the MGs. [15]–[29] MG loss as a result of CL wear has been attributed to CL deposition, mechanical stress, and inflammation. [13], [22] Conversely to these reports, other studies did not find an association between CL wear and disturbances to the MGs. [30]–[36]

In some countries, CLs are considered a regulated medical device and cannot be purchased without a valid prescription from an eye-care practitioner. [37] In Israel and some Asian countries (such as Japan and China) over the counter sales of CLs without a prescription and follow-up are permitted. The rate of ocular complications tends to be higher with CLs acquired through unregulated sources, [37] with the main risk factors being lack of CL fitting and patient education regarding usage, lens hygiene and follow-up care. [37]

The third study compared the prevalence of symptoms and signs of MGD among CL wearers compared with non-CL wearers using the definition of the International Workshop on MGD[1] and objective Cobra HD meibography for assessment of both upper and lower MGs. [93] CL wearers were also analyzed as two separate groups, those that were followed-up by an eye care practitioner (FLU) and those that were not followed-up by an eye care practitioner (non-FLU).

3. State of art

3.1 Meibomian glands (MGs)

MGs are large sebaceous glands, located in the tarsal plates of the upper and lower eyelids. [38] They contain a central duct along the length of the gland [39] with grape-like extensions known as acini. [39] Acini contain modified sebaceous cells called meibocytes [40] that are responsible for the synthesis of the meibum lipid secretion. Meibum secretions generate the lipid layer of tear film, which prevents excessive evaporation of the aqueous layer. [38]

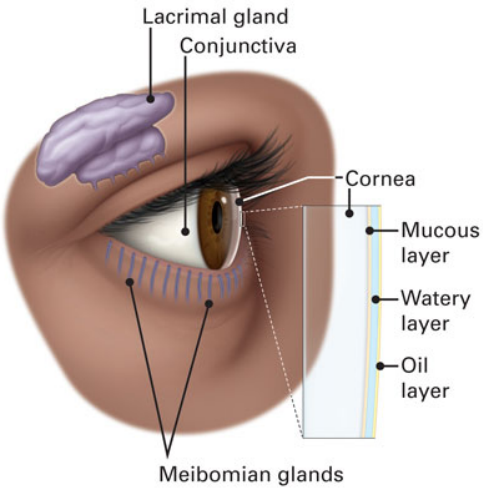


Figure 3.11: Meibomian glands

Large sebaceous glands, located in the tarsal plates of both eyelids. They secrete the meibum which generate the lipid (oil) layer of the tear film *(https://www.aao.org/eye-health/anatomy/meibomian-glands)*

3.2 Meibomian gland dysfunction (MGD)

MGD has been defined by subcommittee of The International Workshop on MGD (2011) as "a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. This may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease''. [1] MGD affects approximately 0.39%-69.3% in the general population worldwide and is one of the primary

causes of evaporative dry eye (DE). [40] The wide range of the prevalence may be due to the lack of a uniform definition, different diagnostic methods and normal variation in incidence of MGD with age and ethnicity. [40]

3.3 Signs

Key signs of MGD include MG loss, altered MG secretion, and morphologic changes in the lids. [41] Eyes with MGD exhibit altered MG secretion that is turbid or cloudy, and tears that are frothy or foamy. [42] Numerous structural changes occur, including thickening, rounding or irregularity of the eyelid, [23] displacement of the mucocutaneous junction, [43] increased vascularity at the lid margin, [44] telangiectasia, [43] madarosis or trichiasis, [44] and notching of the lower lid margin. [42]

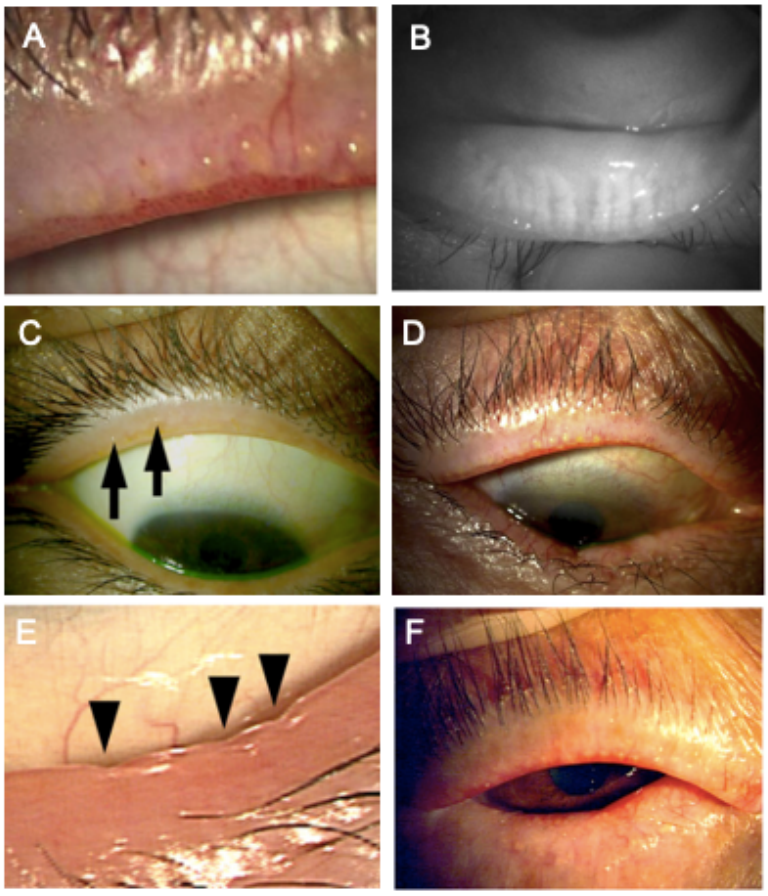


Figure 3.31: MGD Signs

(A) Cloudy meibomian gland secretion (B) meibomian gland loss (C) obstruction of the glands (D) increased vascularity at the lid margin and telangiectasia (E) notching of the lid margin (F) thickening and rounding of the eyelid [45]

3.4 Symptoms

MGD can be symptomatic or asymptomatic. It may be accompanied by ocular symptoms such as fatigue, dryness, grittiness, burning and itching in the eyes. In addition, redness and swelling of the eyelids and occasionally temporary visual blurring. [42]

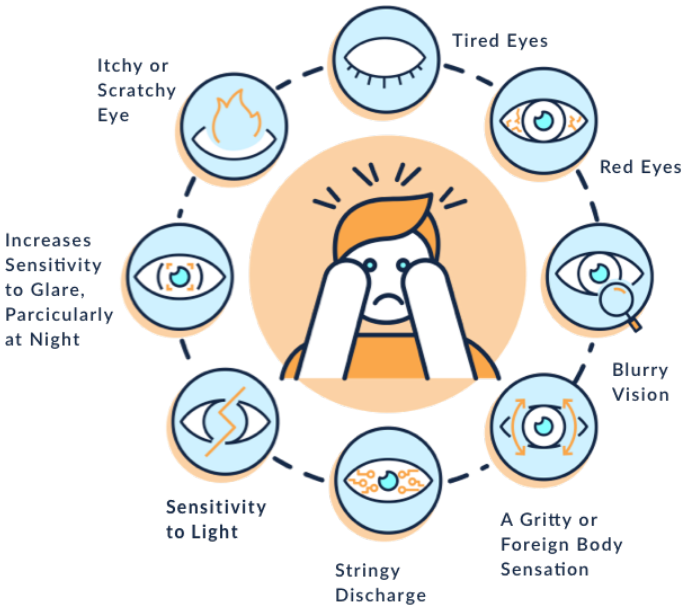


Figure 3.41: MGD symptoms

MGD may be accompanied by ocular symptoms.
(https://eyesonplainville.com/service/optilight-by-lumenis/)

3.5 Pathophysiological mechanisms

Baudouin et al. [46] suggested five separate pathophysiological mechanisms of MGD caused by tear film instability: eyelid inflammation, conjunctival inflammation, corneal damage, microbiological changes and DE disease.

3.6 Diagnosis

In 2011, the International Workshop on MGD created a consensus of diagnostic criteria for the condition. [41] These criteria include a questionnaire based assessment of symptoms, along with functional and morphological

measurements. The functional aspects include meibum expressibility and quality, and tear production (Schirmer test). Tear quality is assessed based on blink rate and interval, tear meniscus height, tear osmolarity, invasive tear-film breakup time, and corneal and conjunctival fluorescein staining. Morphological aspects include quantification of specified lid features and meibography. [41] The Osmoprotection in Dry Eye Disease- Expert Opinion (OCEAN) group updated these diagnostic criteria in 2017 [47] by adding functional diagnostic technologies such as interferometry, non- invasive tear film breakup time measurement, and a morphological assessment using *in vivo* confocal laser microscopy.

3.7 Meibography

Infrared meibographers allow observation [6] and in vivo evaluation of the MG morphology, [7] which is useful for both documentation and follow-up. [48] Several noncontact infrared meibographers are currently available. The OCULUS Keratograph 5 M (OCULUS, Inc., us.oculus.de) [7] employs ImageJ software (imagej. nih.gov) for general image analysis to provide the area of a zone marked by the user. [9] The examiner marks the zones of the everted eyelid and the MGs, and the software provides the area of each zone. The examiner subsequently calculates MG loss by subtraction. [9] However, the two manual steps in this procedure can introduce substantial examiner bias. [7] Despite this, the OCULUS Keratograph 5M has been reported to have good inter-examiner reproducibility (IER; mean difference between examiners of 0.08± 0.55 and 0.13 ± 0.50 grade units in two separate sessions, respectively) with low within-subject variability (WSV; 95% limits of agreement for two different examiners of −1.02 to +1.10 and −1.27 to +1.09 grade units, respectively). [2]

Alternatively, the Cobra HD (csoitalia.it) nonmydriatic digital fundus camera [7] uses Phoenix semi-automated software, which is specially designed for MG analysis [8] and employs a ‘vectorised’ tool with Beziers curves that adapt rapidly to the eyelid shape. [9] Thus, the Phoenix semi-automated software is both quicker and better than the rough segmentation obtained with ImageJ free-hand selection. [9] The Phoenix software requires

that the examiner mark the area of the eyelids and the location where the glands are observed, while the software subsequently calculates the percentage of MG loss. [10] Nevertheless, examiners may vary in their identification of the borders of the tarsus and MGs, leading to inter-observer variability. [6], [11]

The Cobra HD meibographer has been used previously by at least four research groups in the evaluation of MG morphology. [7], [10], [49], [50] In most cases, the standard deviations were quite high with respect to the mean measurement. [10], [49], [50] Pult [10] examined the relationship between age, sex and dry eye, and MG loss was quantified using the Cobra fundus camera with the Phoenix software digital grading tool in 112 participants. In the Pult study [10], the mean MG loss for nondry eye vs. dry eye participants resulted in very high standard deviations; 30±17% and 45±18%, respectively. Similarly, another study of dry eye patients also resulted in a high standard deviation, [50] as was the case for patients with Sjorgen syndrome. [49] By contrast, an additional investigation of patients with dry eyes observed low standard deviations (mean standard deviation for both eyelids = 1.57%). [7] If the high standard deviations previously found reflect instrument variability, then this can impact the results and conclusions. [51] Despite the widespread use of the Cobra HD in research studies and the high standard deviations, the inter-session repeatability (ISR) and inter-examiner reproducibility (IER) of this device have not been examined. These outcomes are important when determining the utility of instruments used in comparative and observational studies, and clinical measurements used to diagnose diseases such as MGD. [4]

Therefore, the second study investigated repeatability and reproducibility of the Cobra HD fundus camera meibographer in participants who were considered either symptomatic or asymptomatic for dry eye based on their Ocular Surface Disease Index (OSDI) questionnaire scores.

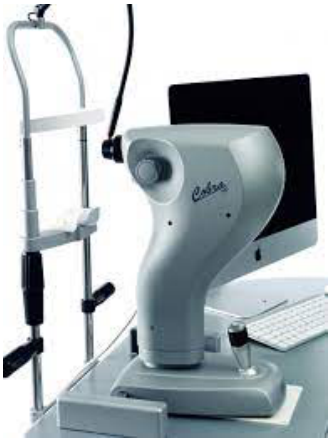


Figure 3.71: Cobra HD meibographer

A nonmydriatic digital fundus camera uses Phoenix semi-automated software, which is specially designed for meibomian gland morphology analysis *(https://www.csoitalia.it/en/prodotto/info/34-cobra)*

3.8 Treatment

The main goal of all MGD treatments is to increase the quality and quantity of the meibomian expression and improve patient's symptoms. The treatments for MGD are recommend according to the severity of the condition, starting with eyelid hygiene, warming and massage, and progressing to the addition of topical lubricants, topical and systemic antibiotics with anti-inflammatory properties, anti-inflammatory agents or omega-3 fatty acid dietary supplementation and topical steroids. [47]

3.9 MGD and Contact lens wear

There are approximately 140–150 million contact lens (CL) wearers worldwide. [15], [52] Approximately 30% to 50% of CL wearers report dry eye (DE) symptoms. [19], [23] The Tear Film & Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) reported that CL wear increases the risk of developing DE by about 2–4 times. [53], [54] CL induced ocular changes leading to DE disease include tear film instability, [19] increased tear evaporation rate and tear osmolarity [33] and decreased tear film meniscus volume. [55] DE and tear film changes in CL wearers are related to reduced visual acuity and decreased wear time, [52] and are a major causative factor for discontinuation of lens wear. [18]

In addition to DE, CL use may induce complications such as keratitis, giant papillary conjunctivitis, infections [19] and corneal disorders. [33], [56], [57] CL wear has also been shown to be correlated with changes in meibomian gland (MG) morphology and function. [15], [18], [23]

A topical literature review was conducted to examine the relationship between contact lens wear and MGD. Of the 115 papers retrieved, 22 articles were included in the review. Studies regarding the relationship between MGD and CL wear are inconclusive, with some demonstrating a significant relationship between MGD and CLs, [15]–[29], and others concluding that there is no significant relationship between them.[30]–[36]

3.91 Pathophysiological mechanisms for MG loss in CL wearers

Several hypotheses have been suggested as causal mechanisms for the loss of MG in CL wearers. Korb and Henriquez [21] and Henriquez and Korb [20] suggested that mechanical obstruction in which epithelial cells accumulate into keratotic clusters, block the meibomian duct, thereby changing the oily secretion. In addition, their results suggest that the presence of bacteria and/or their toxins can damage the MGs. Ong and Larke [26] suggested that permanent rubbing of the CLs at the lid margins during blinking may be a source of mechanical trauma to the eyelids. Arita et al. [19] noted that MG shortening in CL wearers begins from the distal side, indicating that chronic irritation of the MGs by CLs through the conjunctiva is a major causal mechanism for changes in the glands. Uçakhan and Arslanturk-Eren [15] concluded that MG thickening may be due to friction, mechanical irritation, or as a result of primary or secondary inflammatory changes.

3.92 Over the counter sale of CLs

Considering the complications related to CL wear, most governments have treated CLs as medical devices and regulated them accordingly. [37] In most Asian countries (e.g., China) [37] and in some European countries (e.g., Italy), the over the counter (OTC) sale of CLs without a prescription is allowed, while in others (e.g., France), the fitting and the supply of CLs are regulated. [58] In Israel, the marketing of CLs is not regulated yet by law. In 2017, a bill

was passed to regulate the marketing and use of CLs in a manner that will ensure the safe use of CLs and ensure public health but that law has not yet been legislated. As such, this region presents an opportunity to examine complications in CL wearers that attended follow-up visits, those that did not attend to follow-up visit, and non-CL wearing controls.

Several studies report that the complication rate is higher with CLs which are bought from unregulated sources. [59]–[61] The main risk factors include lack of CL fitting and education regarding usage, lens hygiene, prolonged wear of the same CLs (overwear), and follow-up care.[37] Stapleton et al. [62] found that lack of follow-up by eye care practitioners was most common in patients who bought their lenses on the internet. Thus, results of the third study presented herein have legislative implications for public health in our region.

4. Objectives:

Given that there is a lack of consensus about the effect of CL wear on MGD, and the discrepancies between the studies that may underlie the variable findings, this thesis addressed the effect of CL wear on MGD in a prospective study using objective means of assessing MGD, and three experimental cohorts (including a non-CL wearing control group). This global goal was achieved with three sub-studies. The main objectives were:

1. To review the scientific literature regarding the effect of contact lens wear on MGD and to offer an objective approach to address the question in the future. The review compared between studies that reported an effect of CL wear on MGD and studies that did not find an effect of CL wear on MGD.

2. To verify that the Cobra HD fundus camera meibographer can be used to objectively assess the morphology of the MGs by assessing its inter-session repeatability, inter-examiner reproducibility and within-subject variability in young adults with and without symptoms of dry eye.

3. To determine the prevalence of symptoms and signs of MGD by employing two types of subjective, previously validated questionnaires and objective meibography alongside clinical grading scales in the following:

   3A. CL wearers compared with non-CL wearers.

   3B. CL wearers that were followed-up by an eye care practitioner (FLU), CL wearers that were not followed-up (non-FLU), and non-CL wearing controls

4.1 Hypotheses:

1. We hypothesized that based on the literature review, that we would be able to determine if CL wear induces more MGD signs and symptoms.
2. The Cobra HD fundus camera meibography was hypothesized to demonstrate good repeatability and reproducibility due to the similarity

of the analysis software used by the Cobra HD and the Sirius (CSO, Florence, Italy), and the fact that the Sirius has been reported to be a reliable tool for evaluating MGD. [48]

3. More symptoms and signs of MGD were expected to be found among CL wearers compared with non-CL wearers, as previous studies have been shown that CL wear affects the morphology and function of MGs. [15]–[29] Additionally, symptoms and signs of MGD were expected to be higher in non-FLU CL wearers vs. FLU CL wearers, as previous studies show that the rate of ocular complications tends to be higher with CLs acquired through unregulated sources, with the main risk factors being lack of CL fitting and patient education regarding usage, lens hygiene and follow-up care. [37]

5. Study 1: Topical review of the relationship between contact lens wear and meibomian gland dysfunction

Publication: Ifrah R., Quevedo L., Gantz L. (2023) Topical review of the relationship between contact lens wear and meibomian gland dysfunction. *Journal of Optometry*. 16(1):12-19.

5.1 Methods:

A Pubmed database search for research papers written in English between 1980 and 2021 was conducted. Primary search terms and their synonyms were used singly or in combination, including “meibomian gland,” “meibomian gland dysfunction,” “contact lens” and “dry eye", without Boolean operators. Studies whose purpose was to examine the structural and/or functional changes of the MGs in CLs wearers were considered relevant and included. Searches were also performed for articles referenced in bibliographies that were not initially retrieved by the search.

5.2 Results:

The database search resulted in 115 papers, of which 22 were pertinent to the topic of this review. Of these 22 studies, 15 showed an association between MGD and CLs [15]–[29] (Table 5.23) and seven did not [30]–[36] (Table 5.24). Studies tabulated in Tables 1 and 2 used a wide variety of diagnostic criteria, making comparison of the results challenging. Some papers relied on functional assessments alone, while others also used morphological testing, which may better characterize the association of CL wear and MGD. Some papers did not include a control group, which could confound their observations. Thus, the following sections will focus on the studies that used both functional and morphological evaluations and included a control group.

5.21 Studies showing an association between MGD and CLs

Fifteen studies used functional assessment along with morphology [15]–[29] (Table 5.23) and of these, eight included a control group of non-CL

wearers. [15]–[19], [23], [26], [28] Ong and Larke [26] found that rigid, soft, and gas permeable CL wearers who wore their lenses for at least six months, had a higher prevalence of MGD (30%) than non-CL wearers (20%), as assessed by MG expression. There was no significant difference in MGD between the different types of CLs, or between male and female wearers. However, the ages of the participants were not specified. Similarly, a large cohort cross-sectional observational study [19] of rigid and soft CL wearers and non-CL wearers, found that CL wear was significantly associated with a reduced number of functional MGs, with a correlation between wear duration and the number of functional MGs. Furthermore, the average meiboscores of RGP and hydrogel CL wearers were not significantly different, suggesting that the loss of functional MGs does not depend on the CL material. In addition, the average difference between the meiboscores of CL wearers and non-CL wearers was significantly higher in the upper eyelids compared to the lower eyelids. However, they did not assess ocular surface symptoms or MG expressibility. Villani et al. [28] included *in vivo* laser scanning confocal microscopy to assess the morphology of the glands, alongside a subjective DE questionnaire. They observed significantly more morphological changes in MGs among asymptomatic soft hydrogel CL wearers, compared with non-CL wearers. These changes included lower acinar unit diameters, higher glandular orifice diameters, greater secretion reflectivity and greater inhomogeneity of the periglandular interstices. Moreover, the duration of CL wear was significantly correlated to the acinar unit diameters. Additionally, the CL wearers had significantly higher MG loss (dropout). However, the sample size was small and MGs were only evaluated in the lower eyelid, which may under-represent MGD as it affects the upper eyelid more than the lower eyelid. [19] Machalinska et al. [23] assessed MG function (meibum expressibility and quality), MG morphology and dropout (meibography), along with lid margin changes of daily soft CL wearers and non–CL wearer controls. CL use was significantly associated with abnormal meibum quality, lid margin telangiectasia, rounding, notching, hyperemia of the posterior lid margin, orifice plugging and retroplacement. Furthermore, lid margin abnormality and meibum quality scores were significantly correlated with duration of CL wear. The study did not find significant morphological changes of MGs, and did not

find a significant difference in tear film abnormalities between the groups, in contrast with previous studies. [19], [28] Furthermore, a significant difference in subjective ocular symptoms between CL-wearers and controls was not found. This is in contrast to another study that did report a significant difference using the same questionnaire. [28] Alghamdi et al. [18] reported a relationship between MGD and the duration of soft CL wear. They divided CL wearers into short, medium, and long duration of wear, and compared them to previous CL wearers and non-CL wearers. They evaluated both functional and morphological parameters, in both eyelids. MG dropout was assessed with meibography and graded by a scale, while MG function was assessed by quality and quantity of MG expression. They found that all CL wearers had significantly higher rates of MG dropout compared to non-CL wearers. All CL wearers also demonstrated reduced MG expressibility, increased number of plugged orifices, shortening of non-invasive TBUT, and increased MG dropout. These measures did not resolve after a six-month cessation of CL wear, though they did not appear to worsen after two years of wear. Uçakhan and Arslanturk-Eren [15] divided CL wearers into three groups according to the duration of CL wear, comparing them to one another and to controls. MG expressibility was assessed and MG loss was evaluated by meibography. The authors reported that the mean meiboscores of the upper and lower eyelids, percentage of gland loss, and percentage of thickened and curled MGs in both lids were significantly higher in CL wearers compared with the non-CL wearers, while mean TBUT and mean MG expressibility were significantly lower in CL wearers. Silicone hydrogel lenses affected the upper lids mainly in the early years of CL wear. After three years, both lids appeared to be similarly affected. This was the first study to examine and rate MG thickening and curling on tarsal plate structures by meibography, finding that the earliest morphological change is MG thickening of the upper eyelids in CL wearers. Similarly to Villani et al. [28] they also reported that the OSDI score was significantly worse in CL wearers compared with non-CL wearers. This is in contrast to other studies [18], [23] which found no significant differences in OSDI scores between the groups. Gu et al. [16] found significantly higher average total MG dropout and average total distorted MG count in soft CL wearers compared with non-CL wearers. In addition, the duration of CL wear

was significantly correlated with MG dropout, and CL wearers had significantly more DE-related symptoms. MG expression and lid margin morphology were not evaluated. Harbiyeli et al. [17] assessed the condition of the MGs in soft and RGP CL wearers and a control group of non-CL wearers. MG evaluation included an assessment of meibum quality and expressibility. MG morphology was assessed and graded in both eyelids by meibography. Similarly to the results of three other studies, [19], [23], [28] they found that the duration of soft CL use correlated with MG loss in the upper eyelid compared with the control group. Furthermore, those who wore rigid CL materials also had a significantly greater tendency for MG loss. Of note, the rigid CL wearers in this study had keratoconus which can bias the results due to the abnormal ocular surface and different fitting characteristics in this cohort. [17] Moreover, subjects with keratoconus are more likely to suffer from DED and MGD. [63]–[65] The majority of the studies that assessed the impact of CL wear duration on MGD found a significant association between time and MGD severity, [16], [17], [19], [23], [28] for both soft and RGP CLs. [19] This strongly suggests that the length of time patients wear CLs is a significant factor in the development of MGD.

5.22 Studies showing no association between MGD and CLs

Seven studies that found no significant association between MGD and CL wear were identified [30]–[36] (Table 5.24). Six studies [30]–[33], [35], [36] used both functional and morphological assessments. Of these, three included a control group of non-CL wearers. [30], [31], [36] Hom et al. [30] examined randomly selected participants, including both CL wearers and controls. They reported that MGD was significantly correlated with patient age. They found an overall prevalence of 38.9% of MGD, without a significant correlation between CL wear and poor MG expressibility. The study was conducted at several clinical sites, which may have led to non-uniformity among the examiners in diagnosing impaired secretion from the glands. Marren [31] also did not find a significant relationship between MG blockage and CL wear, while investigating the relationship between CL wear, eye make-up use, eye rubbing and MGD. Their MGD evaluation included examination of the MG orifices and assessment of MG expression in CL-

wearing participants compared to non-CL wearers. However, the cohorts were small and their examination included only the lower eyelid. Furthermore, neither study [30], [31] considered CL type and duration of CL wear. The third study that assessed both functional and morphological signs of MGD and that included a control group yielded equivocal results. This multicenter study [36] included non-CL wearers and CL wearers of all CL types and compared MG expressibility and meibum quality in both eyelids, as well as MG dropout, assessed by a meibography. Though higher meiboscores were found to be associated with CL wear, the mean difference of 0.2 was not clinically significant. When the authors controlled for CL wear and several clinical signs such as conjunctival staining and lid wiper epitheliopathy, they found an increased odds of a higher meiboscore in CL wearers. Conversely, OSDI score, TBUT, MG expressibility, meibum quality and tear osmolarity were not found to be associated with CL wear. Therefore, the authors concluded that there is an inconclusive association between CL wear and MG atrophy. However, as stated above, their findings cannot be interpreted as evidence for lack of effect of CL wear on MGs. In addition, the overnight CL wearers and CL dropouts, who are high risk for MG atrophy, were excluded [36], which may limit the conclusions that may be drawn from their study.

Table 5.23: Studies showing association between MGD and CLs. Table summarizing the research studies reporting an association between MGD and CL wear. Columns describe study authors (first column), subjects and controls including their age range (second column) and outcome parameters divided into morphological (columns 3-4), functional (columns 5-13), subjective (column 14), and other measures (last column).

Study	Subjects	Assessment												
		Morphology		Functional									Other	
		Meibography	Slit lamp -lid margin	MG expression	Fluorescein staining	TBUT / NITBUT	Schirmer test	Tear meniscus height	Tear osmolarity	Tear evaporation rate	Lipid layer assessment	Blink rate	Questionnaire	Other
Ong and Larke [26]	CL wearers (N=70) non–CLs wearers (N=70) Age range not specified		✓	✓										Biochemical and physical examination of the MG secretion
Arita et al. [19]	CL wearers (N=121) non–CLs wearers (N=137) age: 16 – 46	✓	✓		✓	✓	✓							
Villani et al. [28]	CL wearers (N=40) non–CLs wearers (N=20) age: 25 – 28	✓	✓	✓	✓	✓	✓						✓	Examination of periglandular inflammation
Machalinska et al. [23]	CL wearers (N= 41)	✓	✓	✓	✓	✓	✓					✓	✓	

	non–CLs wearers (N=31) mean age: 34													
Alghamdi et al. [18]	CL wearers (N=60) non–CLs wearers (N=20) age: 18 – 35	✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	
Uçakhan and Arslanturk-Eren [15]	CL wearers (N=87) non–CLs wearers (N=55) age: 24- 36	✓	✓	✓	✓	✓	✓						✓	MG curling and thickening
Gu et al. [16]	CL wearers (N=85) non-CL wearers (N=63) mean age: CL wearers: 25.52 non-CL wearers: 23.35	✓			✓	✓		✓					✓	
Harbiyeli et al. [17]	CL wearers (N=65) non-CL wearers (N=26) mean age: 33.1	✓	✓	✓	✓	✓	✓						✓	
Korb and Henriquez [21]	CL wearers (N=78) (38 symptomatic vs. 40 asymptomatic)		✓	✓	✓			✓						Cytologic and bacteriologic examination of the lid margin and the MGs

	age: 16 – 82													
Henriquez and Korb [20]	CL wearers (N=50) (38 symptomatic vs. 12 asymptomatic) Age range not specified			✓										Cytologic and bacteriologic examination of the MGs
Mathers and Billborough [24]	CL wearers (N= 42) (27 with giant papillary conjunctivit-is vs. 15 without giant papillary conjunctivit-is) age: 21-50	✓	✓	✓			✓		✓					
Molinari and Stanek [25]	CL wearers (N=105) age: 14 – 58		✓	✓										
Siddireddy et al. [27]	CL wearers (N=30) (17 symptomat-ic vs. 13 asymptoma-tic) age: 18–41	✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	Demodex colonization examination and eyelid sensitivity testing
Llorens-Quintana et al. [22]	CL wearers (N= 41) (33 experience	✓	✓		✓	✓		✓	✓				✓	

	d wearers vs. 8 unexperien-ced wearers) age: 19-29													
Young et al. [29]	CL wearers (N= 274) (226 symptomat-ic vs. 48 asymptom a-tic) mean age (symptoma t-ic): 32.8 mean age (asymptom -atic): 28.7			✓	✓	✓	✓	✓			✓	✓	✓	

Table 5.24: Studies reporting a lack of association between MGD and CLs. Table summarizing research studies reporting a lack of relationship between CL wear and MGD. Columns describe study authors (first column), subjects and controls including their age range (second column), and outcome parameters divided into morphological (columns 3-4), functional (columns 5-13), subjective (column 14), and other measures (last column).

Study	Subjects	Assessment												
		Morphology		Functional									Other	
		Meibography	Slit lamp -lid margin	MG expression	Fluorescein staining	TBUT / NITBUT	Schirmer test	Tear meniscus height	Tear osmolarity	Tear evaporation rate	Lipid layer assessment	Blink rate	Questionnaire	Other
Hom et al. [30]	CL wearers (N=162) non–CLs wearers (N=236) age: <10->60		✓	✓										
Marren [31]	CL wearers (N=20) non–CLs wearers (N=30) age: 22 – 35		✓	✓	✓									
Pucker et al. [36]	CL wearers (N=70) non–CLs wearers (N=70) age: 18 - 43	✓	✓	✓	✓	✓		✓	✓				✓	
Ong [34]	CL wearers (N=81) non–CLs wearers (N=150) age: 15 - 40			✓										
Nichols and Sinnott [33]	CL wearers (N=360) Mean age: 31.1	✓	✓		✓			✓			✓	✓	✓	

Na et al.	CL wearers (N=58) age: 7- 18	✓	✓		✓	✓	✓		✓				✓	Evaluation of inflammation
Pucker et al. [35]	CL wearers (N=112) (56 CL dropout vs. 56 successful CL wearers) age: 18-45	✓	✓	✓		✓							✓	MG width

5.3 Discussion:

The evidence for the effect of CL wear on the MGs is equivocal. In the topical review (study 1), fifteen studies [15]–[29] (Table 5.23) reported functional and/or morphological changes in the MGs among CL wearers, with five studies showing significant correlations between the duration of CL wear and MG loss. [15]–[17], [23], [28]

Conversely, seven studies [30]–[36] (Table 5.24) did not report an association between CL wear and MGD suggesting that CL wear may not increase the risk of MGD. Of these, four found no correlation between CL wear and poor MG expressibility. [30], [31], [34], [36] However, one did find a correlation between CL wear and a higher meiboscore.[36] Another examined overnight orthokeratology which differs greatly from other modalities of CL, and may not be appropriate for assessing the effect of CL wear on MGs. [32] Finally, three other studies, [32], [33], [35] which found no effect of CL use on the MGs, did not include a control group of non-CL wearers, limiting these studies' conclusions.

Discrepancies between studies may be due to differences in the definition of MGD, specifically prior to the 2011 international workshop on MGD. [41] They may also stem from differences in the method of evaluation of MGD. For example, while some studies assess DE symptoms using the Contact Lens Dry Eye Questionnaire (CLDEQ), [18], [27], [29], [33], [35] others used the Dry Eye Questionnaire (DEQ-5), [18] or OSDI questionnaire. [15]–[18], [22], [23], [28], [32] While some studies included a large pool of

participants (≥100) [15], [16], [18], [19], [25], [26], [30], [33], [35], [36] others included small sample sizes [17], [20]–[24], [27], [28], [31] (Table 1,2). Furthermore, studies differ in the inclusion [15]–[19], [23], [26], [28], [30], [31], [34], [36] vs. exclusion [20]–[22], [24], [25], [27], [29], [32], [33], [35] of a control group of non-CL wearers.

Studies also vary in the assessment techniques. For example, MG morphological changes were assessed by transcutaneous infrared MG photography, [24] noncontact infrared meibography, [18], [32], [33] transillumination observation meibography, [28] BG-4M non-contact meibography system, [23] Oculus Keratograph 5M Meibo-Scan, [16], [27], [35], [36] or scheimpflug imaging. [15], [17] Some studies examined only the lower eyelid [20], [21], [23], [24], [28], [30], [31], [33] or only the upper eyelid, [22] while others examined both eyelids. [15]–[19], [27], [35], [36]

Arita et al. [19] reported that the total meiboscores of the upper eyelids were significantly higher compared to the lower lids in CL wearers. They suggested that the upper eyelid might experience more mechanical irritation since it completes larger movements during blinking. Therefore, there may be importance in the examination of both lids.

Another inconsistency between studies is the ages of participants, which may influence the outcomes (see in Tables 5.23 and 5.24). Given that the number of MGs decreases with age, [66] studies that do not control for age, can be misleading.

A further source of discrepancy between studies is the CL materials used by participants of different studies as they may affect the physiology of the MGs as a result of constant mechanical interaction between them. [22] Arita et al. [19] found no significant difference in gland atrophy area between rigid gas permeable and hydrogel CLs wearers, while Llorens-Quintana et al. [22] found significant changes in the area of gland atrophy and the number of glands of hydrogel CL wearers as opposed to silicone hydrogel CL wearers. Other studies [20], [21], [30] did not consider CL materials or duration of CL wear, parameters that may affect the results.

Studies conducted in different countries with different ethnic populations (Malaysia,[34] Los Angeles,[30] Spain,[22] Australia,[27] Turkey,[15] Japan[19]) may also account for differences in reported outcomes. For example, Asians have an absent or lower crease and more fat in their upper eyelids, [67] and produce more secretion from the MGs upon expression compared to Caucasians. [34]

Additionally, some studies were multicenter clinical trials [17], [29], [35], [36] that may be limited by inter-examiner variation in methodology, evaluation and rating of clinical findings.

The results of the studies in Table 5.23 suggest several pathophysiological mechanisms for loss of MG in CL wearers. Korb and Henriquez [21] and Henriquez and Korb [20] suggested that mechanical obstruction in which epithelial cells accumulate into keratotic clusters, block the meibomian duct, thereby changing the oily secretion. In addition, their results suggest that the presence of bacteria and/or their toxins can damage the MG. Ong and Larke [26] suggested that the permanent rubbing of the CLs at the lid margins during blinking may be a source of mechanical trauma to the lids. Arita et al. [19] noted that MG shortening in CL wearers began from the distal side, indicating that chronic irritation of the MGs by CLs through the conjunctiva is a major causal mechanism for changes in the glands. Uçakhan et al. [15] concluded that MG thickening may be due to friction, mechanical irritation, or as a result of primary or secondary inflammatory changes. Further research is required to elucidate the exact pathway and it may be a combination of mechanical obstruction, microbiological changes and mechanical abrasion by the CL that cause MGD.

One limitation of this review is the fact that only publications between 1980-2021 were included and it is possible that more studies were published since which potentially could have provided further insights as to the relationship between contact lens wear and MGD. However, only two such studies were identified in a search conducted in April 2023 and it is safe to assume that they would not have greatly impacted the conclusions of our investigation.

Conclusions

Based on our review of the current literature, the effect of CL wear on the MGs is ambiguous and requires further elucidation. Prospective, longitudinal, large cohort, controlled, randomized studies are required to better understand the mechanisms of changes in MG morphology and function of CL wearers. Efforts should be made to include several CL materials, and CL wear-durations, with analysis taking these parameters into account. Furthermore, new studies should adopt the same criteria and techniques to diagnose MGD, such as those suggested by the international workshop on MGD [15] or OCEAN group [47] and include a control group. In so doing, these studies will efficiently and effectively identify the long-term impact of CLs on MG and MGD.

6. Study 2: Repeatability and reproducibility of Cobra HD fundus camera meibography in young adults with and without symptoms of dry eye

Publication: Ifrah R., Quevedo L., Gantz L. (2023) Repeatability and reproducibility of Cobra HD fundus camera meibography in young adults with and without symptoms of dry eye. *Ophthalmic and Physiological Optics*. 43(2):183-194.

6.1 Methods:

The study complied with the ethical principles of the Declaration of Helsinki and commenced after approval from the internal ethics committee of Hadassah Academic College. The methods were explained verbally and participants signed a statement of informed consent prior to their participation.

6.11 Subjects:

Participants in this prospective study were recruited through advertisements posted to the student body at Hadassah Academic College (HAC). Those suffering from systemic disease, ocular infection, ocular inflammation or allergies were excluded, as were individuals taking medication that might affect ocular surface symptoms and clinical characteristics (including MG). Pregnant or lactating women were excluded. Only participants up to 45 years of age were included to avoid age-related confounding effects, as age is a significant risk factor for MGD. [66], [68], [69] Contact lens wearers were also excluded as an association between MGD and contact lens wear has been demonstrated. [12]

All examinations took place in the contact lens clinic at the Department of Optometry, HAC, in a designated examination room.

Seventy-four participants were measured on the same day by two examiners (Examiner 1, Examiner 2) to determine the IER. Of these, 66 were re-examined by Examiner 1 on a second date to calculate the ISR. An additional six participants were examined by Examiner 1 on two different dates. Their

data were included with the 66 participants described above, yielding a total of 72 participants in the ISR experiment (Figure 6.12).

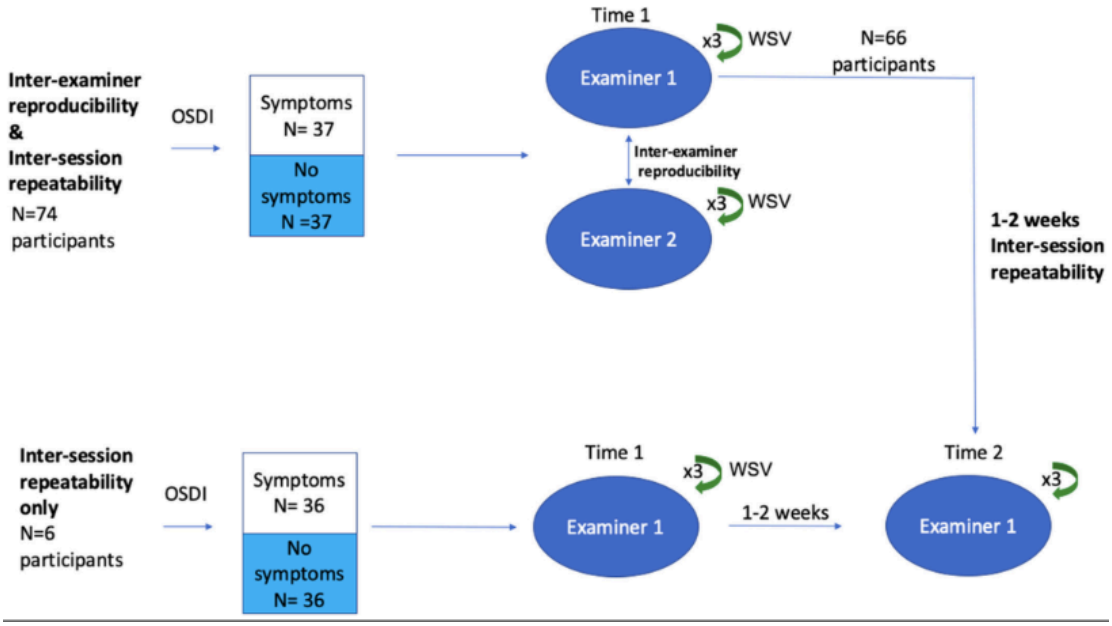


Figure 6.12: Flow chart of the Research Methodology

Flowchart of the experimental procedures as described in the text. 74 participants were measured on the same date by two different examiners (Examiner 1, Examiner 2) to determine the inter-examiner reproducibility. Of these 74 participants, 66 were re-examined by Examiner 1 on a second date to calculate the inter-session repeatability (ISR). An additional six participants were examined only by Examiner 1 on two different dates, and their data were included with the 66 participants described above, yielding a total of 72 participants in the ISR experiment. Green arrows represent three consecutive measurements from the same participant that were used to determine within-subject variability (WSV). The Ocular Surface Disease Index (OSDI) questionnaire scores classified participants into symptomatic (≥13) and asymptomatic (no symptoms, scores < 13).

6.13 Experimental procedures:

Exclusion criteria were verified based on the medical and ocular history. The OSDI questionnaire was administered to the participants, as it is a widely used questionnaire for dry eye clinical trials. [70] Participants with OSDI scores ≥13 or <13 were classified as symptomatic and asymptomatic, respectively. [70]

The morphology of the upper and lower eyelids can differ, [38] and some studies have reported variations in MG loss from the upper and lower eyelids in patients with MGD. [7], [48], [71]–[74] Thus, this investigation compared data from the upper and lower eyelids separately. MGs of the right eyes of the participants were imaged using the Cobra HD fundus camera meibographer, which includes a charge-coupled device high-resolution sensor (2448 × 2051 pixels [5 MPixel]) and an infrared light emitting diode at 850 nm. [7] The upper and lower eyelids were everted while the participant remained stable. After the MGs were seen in focus, the examiner ensured that the lacrimal points were visible and captured three consecutive images per eyelid such that the images were of equivalent magnification. Thus, a total of six images were measured for each participant. Examiners selected and marked both the total area of the everted eyelid and the area containing MGs using the 'vectorised' tool with Beziers curves that conformed to the shape of the eyelid (Figure 6.14). Then, the Phoenix software improved the contrast [6] and automatically measured the percentage and grade of MG loss according to the meiboscale grading system [9] based on Pult and Nichols. [75] The meiboscale characterizes the percentage of MG loss (0%–100%) such that 0, 1, 2, 3 and 4 represent no loss, <25% loss, 26%–50% loss, 51%–75% loss and >75% loss, respectively. [9]

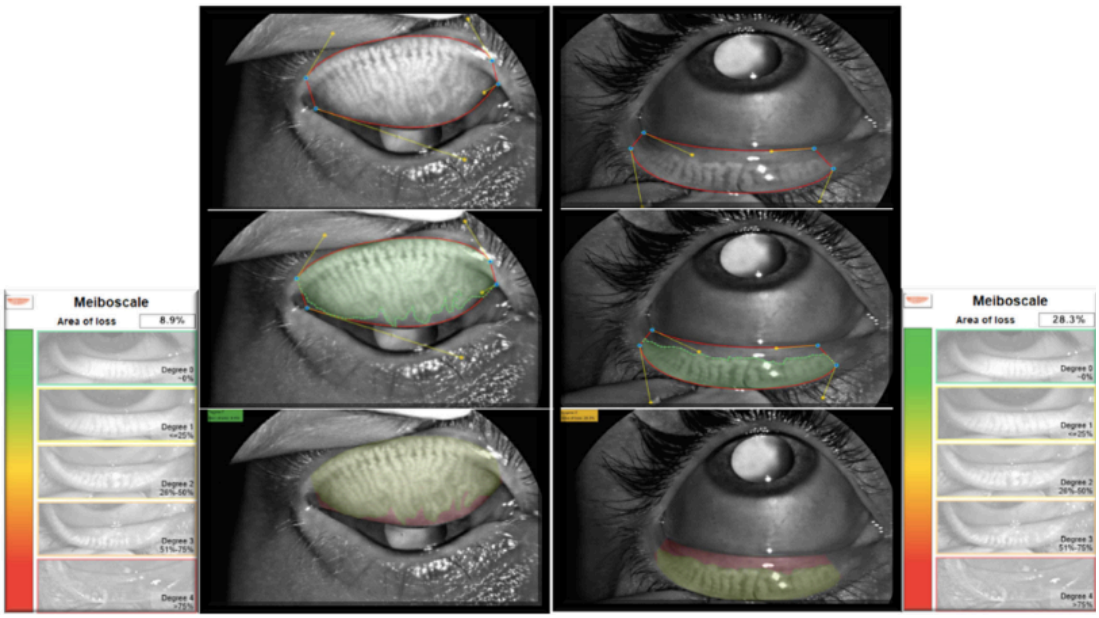


Figure 6.14:

Analysis of meibomian gland (MG) loss area by the Phoenix software of the upper (left) and lower eyelid (right). Top images: Total MG area measurement. Middle images: Region of interest containing MGs. Bottom images: Calculation of meibomian gland area loss (degree: 0–4, scale: 0%–100%).

Inter-session repeatability

As shown in Figure 6.12, participants in this sub-study (*N* = 72) were retested 1–2 weeks after their initial experimental session at approximately the same time of day in the same designated examination room by Examiner 1.

Inter-examiner reproducibility

As shown in Figure 6.12, participants in this sub-study (*N* = 74) were measured by two examiners during the same experimental session. Participants were measured in a counter-balanced design. Thus, if Examiner 1 was the first to measure the first participant, Examiner 2 was the first to measure the second participant and so on. The examiners were masked as to each other's findings.

Within-subject variability

The WSV was determined from three consecutive measurements of the same eyelid captured by each examiner.

6.2 Statistical Analysis:

Sample size

Sample size was calculated per the formulae recommended by McAlinden et al. [76] for three repeated measurements. Sample sizes of 43 and 24 participants were required for confidence in the estimate of 15% and 20%, respectively. Thus, with 72 participants, the experiment was sufficiently powered. For the correlation and Bland and Altman analyses, based on Cesana and Antonelli [77] for a power of 0.95, it was necessary to measure 12 subjects for correlation coefficients of 0.85. As our correlation coefficients

were all >0.86 and there were 72 pairs of measurements, this study was sufficiently powered.

Demographic data were evaluated using descriptive statistics. The mean and standard deviation of the percentage of MG loss from the participants' right eyes were calculated. The normality of the outcome measures was assessed using the Kolmogorov–Smirnov test. Data were analyzed for the entire sample and, for symptomatic and asymptomatic subgroups separately. Differences between the subgroups were examined for significance using a Mann–Whitney *U*-test that was applied due to a data set that was not normally distributed.

Differences between the upper and lower eyelids were examined using correlation analysis and the Friedman test due to a data set that was not normally distributed.

Differences were considered statistically significant when $p < 0.05$. Statistical analysis was performed with Microsoft Office Excel (microsoft.com) and IBM SPSS Statistics (version.27, ibm.com).

Inter-session repeatability and inter-examiner reproducibility

The ISR and IER were compared using the Friedman test due to non-normal distribution of the outcome measures from the lower eyelids. Both repeatability and reproducibility were determined by calculating the square root of the mean square within groups (Sw), the repeatability limit that is determined by multiplying the Sw by $1.96\sqrt{2}$ (2.77), which provides an estimate of the limits within which 95% of measurements should lie. [78], [79] The intra-class correlation coefficient (ICC) and the 95% confidence intervals (CI) for the ICC were used to determine the IER. [48], [80] The ICC ranges from 0–1, with values closer to 1 representing better consistency of measurements. [81]

Pearson's correlation was applied to normally distributed data, and Spearman's correlation was applied to non-normally distributed data. The Bland and Altman analysis [82] was applied to data sets that were

significantly correlated. In this analysis, the differences between the measurements of the two sessions or two examiners were plotted against the mean measurement for each subject. It is expected that 95% of differences fall within two standard deviations or less. [82] The mean difference represents the bias, which should be close to zero. [83]The limits of agreement represent the coefficient of repeatability [84] for all comparisons.

Clinically, measurements within 12.5% of one another are considered to be identical, as this is half of the minimum step size of 25% discriminating between the grades of the meiboscale. [85]

Within-subject variability

The WSV was determined by calculating the standard deviation of three consecutive measurements, the Sw and 2.77 Sw of the three consecutive measurements. In addition, the ICC values and the 95% CIs for the ICC were reported. [86]

6.3 Results:

6.31 Inter-session repeatability

Seventy-two participants (mean age: 23.0 ± 4.5 years, range: 19–43, 57 female), 36 symptomatic and 36 asymptomatic were recruited for the ISR experiment. The demographic data of these participants are shown in Table 6.32.

Table 6.32: Demographic data of participants in the inter-session repeatability study

	All participants	**Symptomatic**	**Asymptomatic**
N	72	36 (50.0%)	36 (50.0%)
Female	57 (79.2%)	30 (83.3%)	27 (75.0%)
Mean age ±SD (years)	23.0±4.5	22.0±2.4	23.9±5.8

Age range (years)	19-43	19-30	19-43

The mean MG loss (%) from both sessions and both eyelids for all participants and the symptomatic and asymptomatic subgroups are tabulated in Table 6.33. The MG loss range was 0.6%–49.3% and 0.3%–33.7%, in the upper and lower eyelids, respectively.

Table 6.33: Mean MG loss (%) in first and second experimental sessions
The table displays the means and standard deviations of the mean MG loss (%) measurements of session 1 (left section) and session 2 (right section), as well as the range of measurements for the upper (first and third column) and lower eyelids (second and fourth column), for all participants (upper rows), symptomatic participants (middle rows), and asymptomatic participants (bottom rows)

Mean MG loss (%)		**Session 1**		**Session 2**	
		Upper eyelid	**Lower eyelid**	**Upper eyelid**	**Lower eyelid**
All Participants N=72	**Mean ± SD**	13.5±9.5	7.5±6.9	12.8±8.5	7.3±6.3
	Range	0.6-49.3	0.3-33.7	0.7-45.8	0.3-29.6
Symptomatic N=36	**Mean ± SD**	14.5±10.4	8.2±7.4	14.3±9.7	8.0±6.6
	Range	1.8-49.3	0.3-31.1	1.8-45.8	0.3-28.6
Asymptomatic N=36	**Mean ± SD**	12.5±8.6	6.7±6.4	11.3±6.9	6.7±5.9
	Range	0.6-39.9	0.6-33.7	0.7-24.9	0.7-29.6

The MG loss of the upper eyelids was normally distributed, whereas the MG loss in the lower eyelids as recorded by one examiner was not normally distributed. Therefore, Pearson correlation analysis was used to determine the correlation in the upper eyelids, while Spearman correlation analysis was

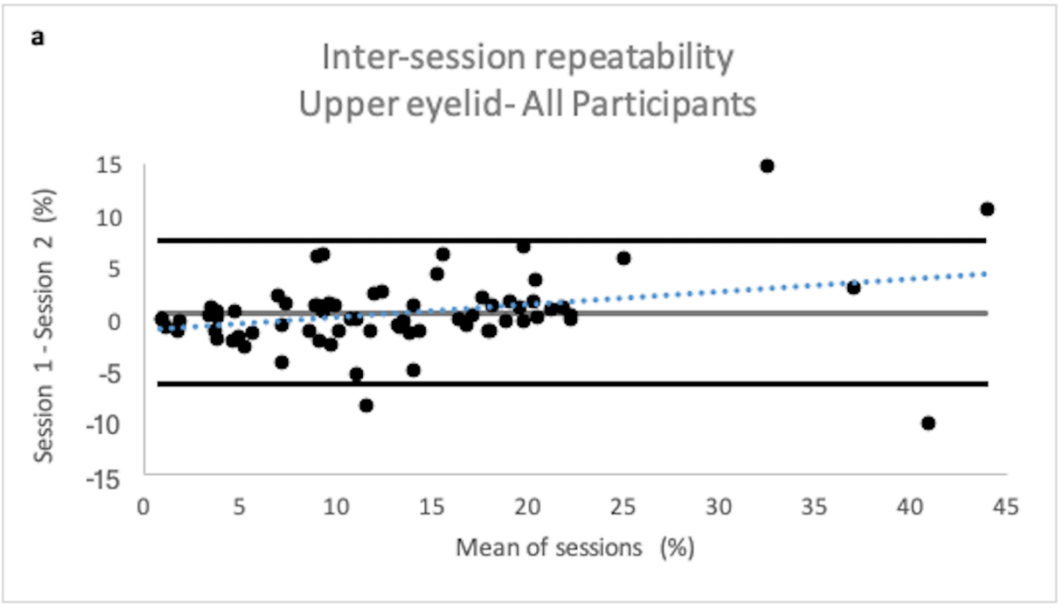
used in the lower eyelids. The MG loss of the upper and lower eyelids was positively correlated ($p<0.001$ for all conditions, Table 6.34) and not significantly different (Friedman test; Table 6.34, upper eyelids, p=0.11, 0.30, 0.32, lower eyelids, p=0.81, 0.74, 1.00, for all participants, symptomatic and asymptomatic subgroups, respectively) between the first and second sessions. Meibomian gland loss did not vary significantly between the asymptomatic and symptomatic subgroups (Mann– Whitney U- test), for Examiner 1 (upper eyelids, p=0.23, lower eyelids, p=0.21) or Examiner 2 (upper eyelids, p=0.22, lower eyelids, p=0.20). The mean difference that represents the bias [83] and limits of agreements that represent the coefficient of repeatability [87] for all comparisons are tabulated in Table 6.34 together with Sw and 2.77 Sw. The mean differences (md) between the sessions were 1.20% and below for the entire cohort, asymptomatic and symptomatic subgroups for both upper and lower eyelids. 90.28% and 93.06% of the observations fell within the limits of agreement, in the upper and lower eyelids, respectively. The Bland and Altman analysis is shown in Figure 6.35. Only one participant had a difference between the measurements >12.5% in the upper eyelids (99% of the participants had differences in the measurements that were lower than 12.5%), and none of the participants had differences >12.5% in the lower eyelids.

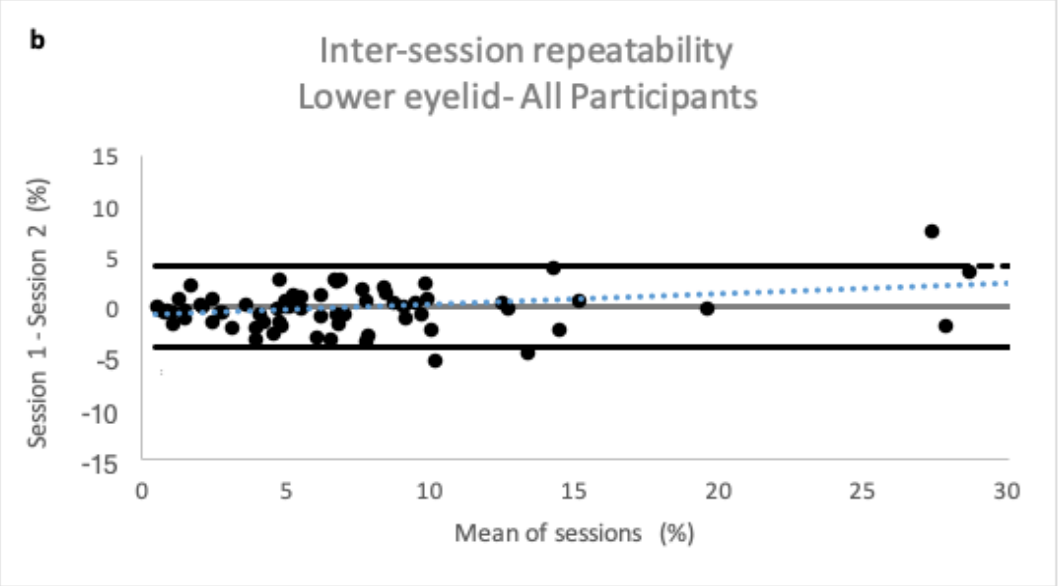
Upper eyelid								
	R	**P**	**Mean difference (%) [Range]**	**P**	**Upper 95% CI**	**lower 95% CI**	**Sw**	**2.77Sw**
All participants (N=72)	0.93	<0.001	0.68±3.54 [-9.93 - 14.97]	0.11	7.62	-6.25	1.26	3.50
Symptomatic (N=36)	0.95	<0.001	0.18±3.35 [-9.93-10.67]	0.30	6.75	-6.40	1.18	3.26
Asymptomatic (N=36)	0.91	<0.001	1.19±3.69 [-5.23-14.97]	0.32	8.43	-6.05	1.34	3.72
Lower eyelid								

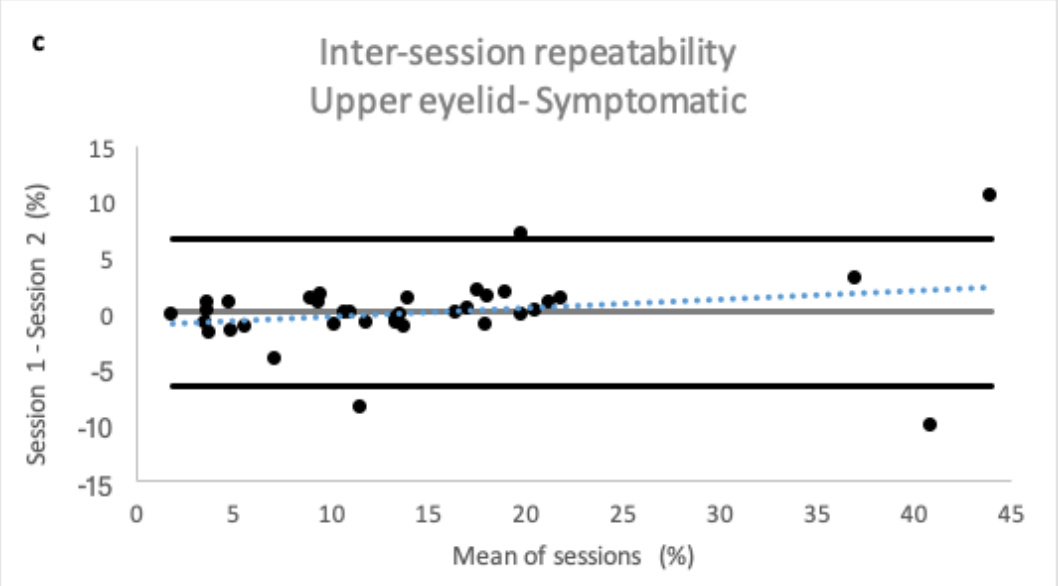
	Rho	P	Mean difference (%) [Range]	P	Upper 95% CI	lower 95% CI	Sw	2.77Sw
All participants (N=72)	0.89	0	0.12±2.07 [-5.17 - 7.57]	0.81	4.17	-3.94	1.04	2.88
Symptomatic (N=36)	0.86	0	0.27±2.12 [-3.33-7.57]	0.74	4.42	-3.88	1.05	2.91
Asymptomatic (N=36)	0.91	0	-0.04±2.04 [-5.17-4.13]	1.00	3.97	-4.04	1.03	2.86

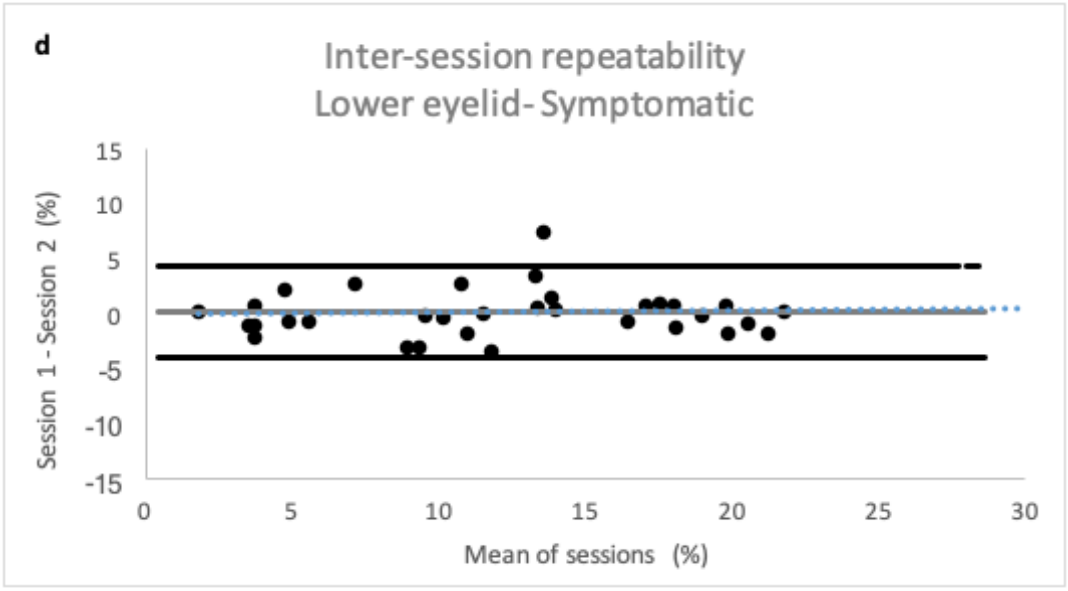
Table 6.34: Statistical outcome measures of inter-session repeatability. The correlation coefficient (Pearson's R for upper eyelids and Spearman's Rho for lower eyelids, first column), p-value of the correlation analysis (second column), mean difference between the first and second session measurements (third column), p-value of the Friedman test comparing between the measurements of the first and second sessions (fourth column), upper (fifth column) and lower (sixth columns) limits of agreement, the square root of the mean square within groups (Sw, seventh column), and the repeatability limit within which 95% of the measurements should be ($1.96\sqrt{2}$ (2.77Sw), eighth column), of the measurements of the first and second sessions.

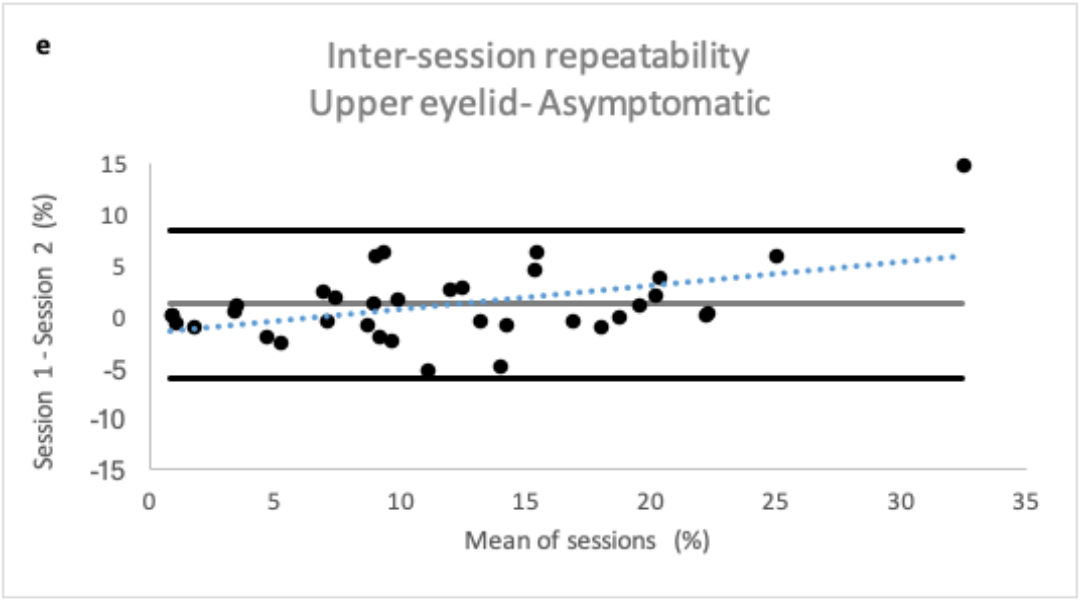
Figure 6.35: Bland and Altman analysis of inter-session repeatability: Bland and Altman plots of inter-session repeatability, representing the mean difference in MG loss in the upper and lower eyelids, for all participants and for symptomatic and asymptomatic subgroups. The gray line represents the mean difference (bias), the black lines show the 95% limits of agreement, and dotted blue lines represent the trendlines fit to the data. Each data point represents one participant. All values presented are in %. (a) Upper eyelid, all participants (b) Lower eyelid, all participants (c) Upper eyelid, symptomatic participants (d) Lower eyelid, symptomatic participants (e) Upper eyelid, asymptomatic participants (f) Lower eyelid, asymptomatic participants

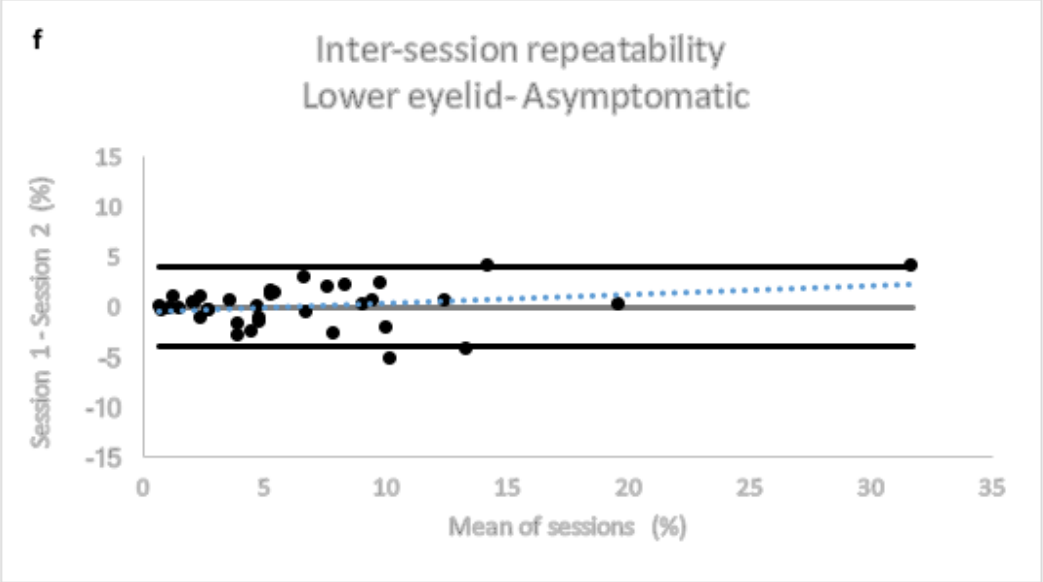



b
Inter-session repeatability
Lower eyelid- All Participants
Session 1 - Session 2 (%)
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
30
Mean of sessions (%)


c
Inter-session repeatability
Upper eyelid- Symptomatic
Session 1 - Session 2 (%)
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
30
35
40
45
Mean of sessions (%)


d
Inter-session repeatability
Lower eyelid- Symptomatic
Session 1 - Session 2 (%)
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
30
Mean of sessions (%)


e
Inter-session repeatability
Upper eyelid- Asymptomatic
Session 1 - Session 2 (%)
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
30
35
Mean of sessions (%)



6.36: Inter- examiner reproducibility

Seventy-four participants (mean age: 23.4 ± 4.9 years, range: 19–43, 56 female), 37 symptomatic and 37 asymptomatic (Table 6.37) were included in the IER experiment.

Table 6.37: Demographic data of participants in the inter-examiner reproducibility study

	All participants	Symptomatic	Asymptomatic
N	74	37 (50%)	37 (50%)
Female	56 (75.7%)	31 (83.8%)	25 (67.6%)
Mean age ±SD	23.4±4.9	22.5±3.5	24.2±5.9
Age range	19-43	19-37	19-43

The mean MG loss (%) measured in both eyelids by both examiners for all participants and the symptomatic and asymptomatic subgroups are tabulated in Table 6.38. The MG loss range was 0.7%–45.8% and 0.3%–29.6%, in the upper and lower eyelids, respectively.

Table 6.38: Mean MG loss (%) measured by both examiners
The table displays the means and standard deviations of the mean MG loss (%) measurements of examiner 1 (left section) and examiner 2 (right section), as well as the range of measurements for the upper (first and third column) and lower eyelids (second and fourth column), for all participants (upper rows), symptomatic participants (middle rows), and asymptomatic participants (bottom rows)

Mean MG loss (%)		Examiner 1		Examiner 2	
		Upper eyelid	Lower eyelid	Upper eyelid	Lower eyelid
All Participants (N=74)	**Mean ± SD**	12.7±8.2	7.0±6.2	13.1±8.0	7.4±6.2
	Range	0.7-45.8	0.3-29.6	0.5 –39.7	0.2-27.7
Symptomatic (N=37)	**Mean ± SD**	14.4±9.4	7.9±6.6	14.6±9.0	8.0±6.3
	Range	1.8-45.8	0.3-28.6	0.5-39.7	0.3-27.7
Asymptomatic (N=37)	**Mean ± SD**	11.1±6.6	6.1±5.8	11.6±6.6	6.8±6.1
	Range	0.7-24.9	0.7-29.6	1.0-25.5	0.2-25.7

The MG loss of the upper eyelids was normally distributed, whereas the MG loss of the lower eyelids was not normally distributed. Therefore, Pearson correlation analysis was used to determine the correlation between the examiners for the upper eyelids, while Spearman's correlation analysis examined the relationship between the MG loss of the lower eyelids of the two examiners. The MG loss of the upper and the lower eyelids was positively correlated between the examiners for all conditions ($p < 0.001$) and were not significantly different (Friedman test; Table 6.39, upper eyelids, p=0.32, 0.41, 0.14, lower eyelids, p=0.10, 0.14, 0.40, for all participants, symptomatic and asymptomatic sub-groups, respectively). The mean difference and limits of agreements for all comparisons are tabulated in Table 6.39 together with Sw, 2.77 Sw and ICC values. The Bland and Altman analysis can be seen in Figure 6.4. The md between the examiners were <0.72% for the entire cohort,

asymptomatic and symptomatic subgroups for both upper and lower eyelids. Of the observations, 94.60% fell within the limits of agreement in either eyelid. All participants had differences in measurements between the examiners that were <12.5% for both the upper and lower eyelids.

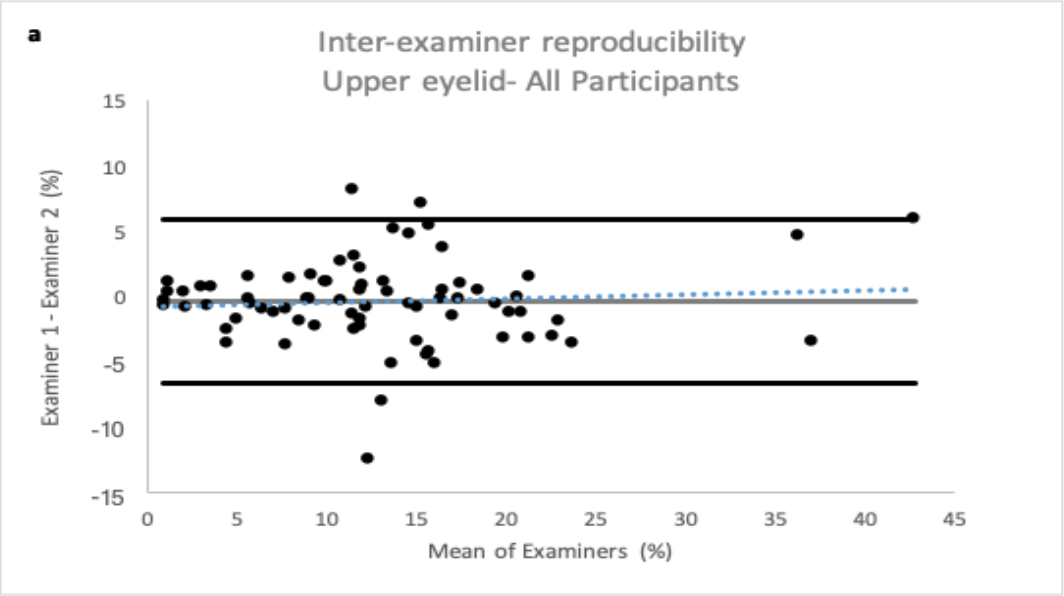
Table 6.39: Statistical outcome measures of inter-examiner reproducibility. The correlation coefficient (R, first column), p-value of the correlation analysis (second column), mean difference between the measurements of the two examiners (third column), p-value of the test comparing the measurements of the two examiners (Friedman test for lower eyelids, fourth column), upper (fifth column) and lower (sixth columns) limits of agreement, the square root of the mean square within groups (Sw, seventh column), the repeatability limit within which 95% of the measurements should be (1.96√2 (2.77Sw), eighth column) and the intraclass correlation coefficient (ICC) values (ninth column) between the measurements of the two examiners.

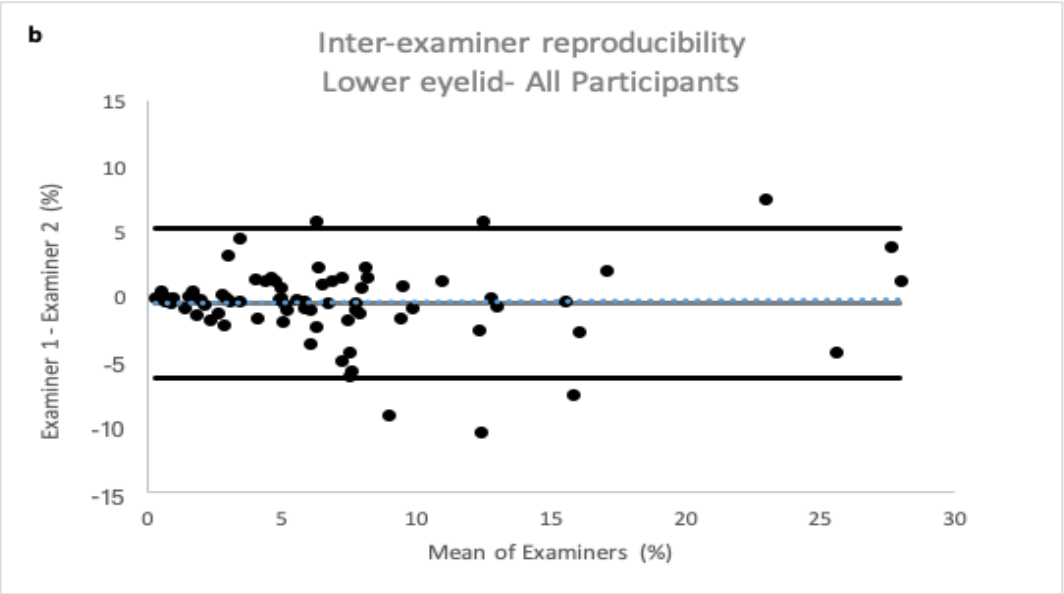
Upper eyelid									
	R	**P**	**Mean difference (%) [Range]**	**P**	**Upper 95% CI**	**lower 95% CI**	**Sw**	**2.77Sw**	**ICC [95% CI]**
All participants (N=74)	0.92	<0.001	-0.37±3.20 [-12.47-8.13]	0.32	5.90	-6.65	1.27	3.51	0.92 [0.88-0.95]
Symptomatic (N=37)	0.94	<0.001	-0.25±3.20 [-8.00-8.13]	0.41	6.02	-6.51	1.30	3.59	0.94 [0.89-0.97]
Asymptomatic (N=37)	0.88	<0.001	-0.50±3.24 [-12.47-7.13]	0.14	5.86	-6.86	1.24	3.43	0.88 [0.78-0.94]
Lower eyelid									
	Rho	**P**	**Mean difference (%) [Range]**	**P**	**Upper 95% CI**	**lower 95% CI**	**Sw**	**2.77Sw**	**ICC [95% CI]**
All participants	0.82	0	-0.42±2.92 [-10.27-7.53]	0.10	5.31	-6.15	1.16	3.23	0.89 [0.83-0.93]

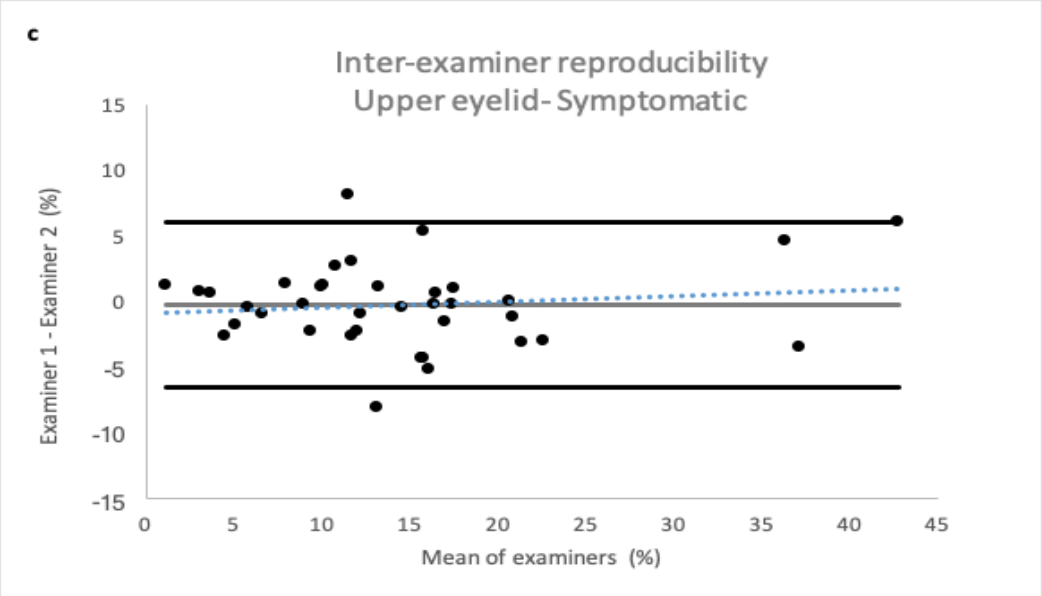
(N=74)									
Symptomatic (N=37)	0.77	0	-0.12±2.68 [-6.03-7.53]	0.14	5.13	-5.38	1.14	3.16	0.91 [0.84-0.96]
Asymptomatic (N=37)	0.86	0	-0.72±3.16 [-10.27-5.90]	0.40	5.47	-6.91	1.19	3.28	0.86 [0.74-0.93]

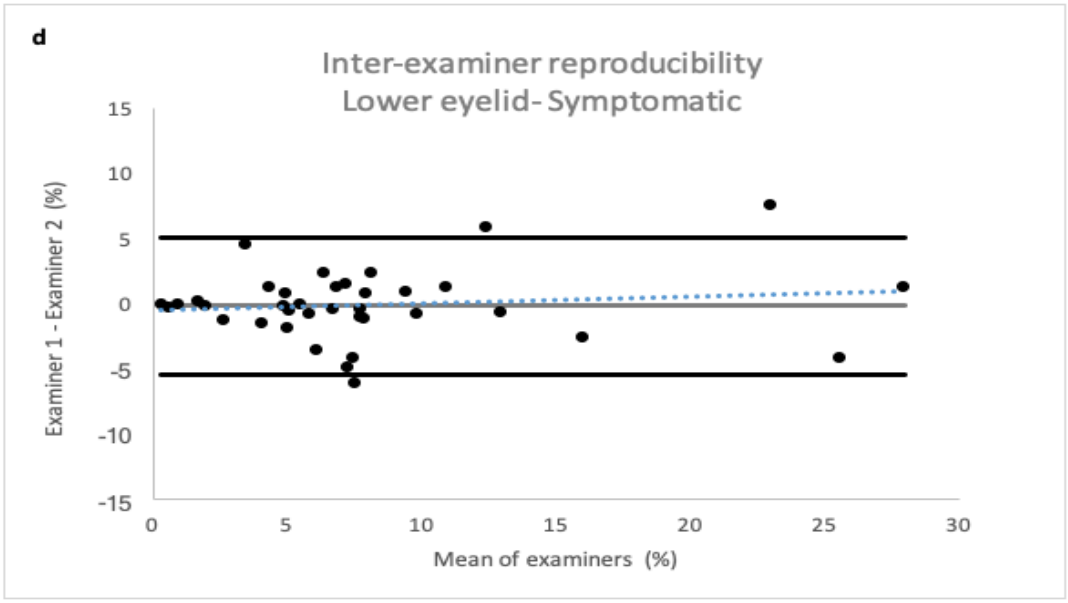
Figure 6.4: Bland and Altman analysis of inter-examiner reproducibility:

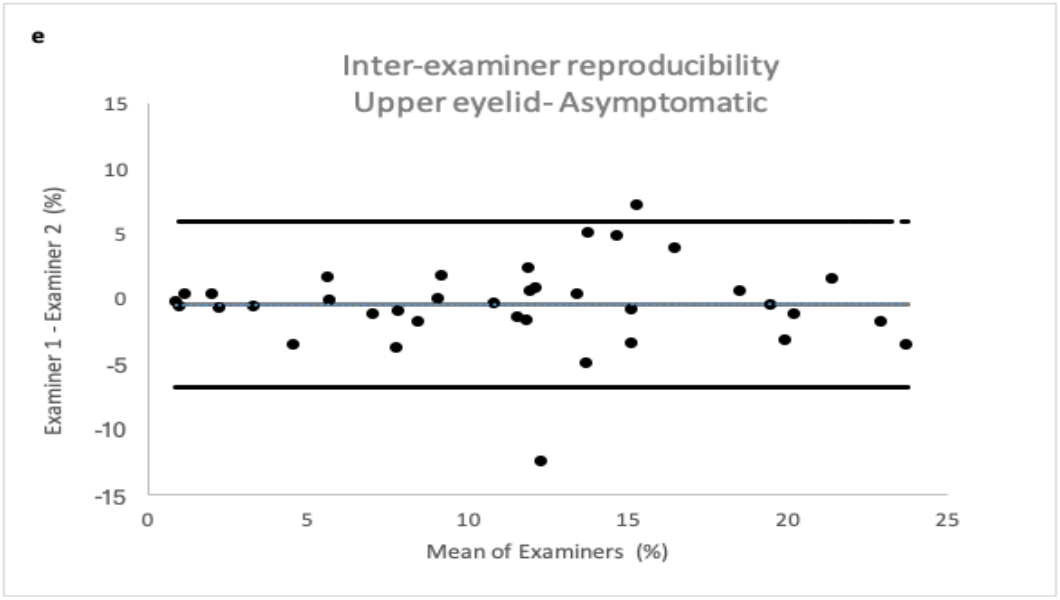
Bland and Altman plots of inter-examiner reproducibility, representing the mean difference in MG loss in the upper and lower eyelids, for all participants and for symptomatic and asymptomatic subgroups. The grey line represents the mean difference (bias), the black lines show the 95% limits of agreement, and dotted blue lines represent the trendlines fit to the data. Each data point represents one participant. All values presented are in %. (a) Upper eyelid, all participants (b) Lower eyelid, all participants (c) Upper eyelid, symptomatic participants (d) Lower eyelid, symptomatic participants (e) Upper eyelid, asymptomatic participants (f) Lower eyelid, asymptomatic participants

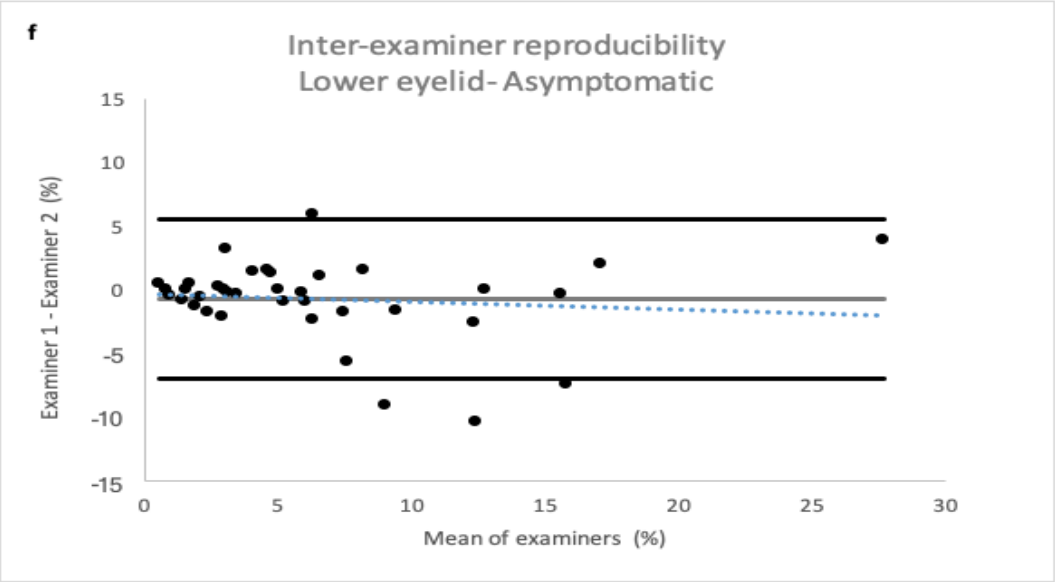



b
Inter-examiner reproducibility
Lower eyelid- All Participants
Examiner 1 - Examiner 2 (%)
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
30
Mean of Examiners (%)


c
Inter-examiner reproducibility
Upper eyelid- Symptomatic
Examiner 1 - Examiner 2 (%)
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
30
35
40
45
Mean of examiners (%)


d
Inter-examiner reproducibility
Lower eyelid- Symptomatic
Examiner 1 - Examiner 2 (%)
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
30
Mean of examiners (%)


e
Inter-examiner reproducibility
Upper eyelid- Asymptomatic
Examiner 1 - Examiner 2 (%)
15
10
5
0
-5
-10
-15
0
5
10
15
20
25
Mean of Examiners (%)



6.41 Within- subject variability

The WSV of the measurements of Examiner 1, for both eyelids and sessions, had Sw values ≤1.27% and ICC values ≥0.93 for all subgroups (Table 6.42), indicating low WSV. The WSV of the measurements of Examiner 2, for both eyelids and sessions, had Sw values ≤1.34% and ICC values ≥0.89 for all subgroups (Table 6.42), also indicating low WSV.

Table 6.42: Statistical outcome measures of within subject variability and correlations between Ocular Surface Disease Index questionnaire scores and mean meibomian gland loss. The square root of the mean square within groups (Sw, third column), and the repeatability limit within which 95% of the measurements should lie (1.96√2 (2.77Sw), fourth column), intraclass correlation coefficient (ICC) values (fifth column) of the measurements, correlation coefficients (Pearson's R for upper eyelids and Spearman's Rho for the lower eyelids), and p-values (P) of the correlations of the upper (Left section) and lower eyelids (Right section) for each examiner are tabulated. Blue cells represent significant correlations

		Upper eyelid					Lower eyelid				
		Sw	**2.77Sw**	**ICC [95% CI]**	**R**	**P**	**Sw**	**2.77Sw**	**ICC [95% CI]**	**R**	**P**
Examiner 1 Session 1	All participants (N=72)	1.15	3.18	0.96 [0.94-0.97]	0.14	0.25	1.04	2.89	0.95 [0.92-0.97]	0.24	0.05
	Symptomatic (N=36)	1.27	3.51	0.96 [0.93-0.98]	0.05	0.76	1.10	3.04	0.96 [0.93-0.98]	0.18	0.30
	Asymptomatic (N=36)	1.01	2.80	0.97 [0.94-0.98]	0.27	0.11	0.98	2.72	0.93 [0.89-0.96]	0.34	0.06

Examiner 1 Session 2	All participants (N=74)	1.17	3.25	0.97 [0.95-0.98]	0.15	0.17	1.09	3.02	0.95 [0.93-0.97]	0.26	0.02
	Symptomatic (N=37)	1.19	3.31	0.96 [0.93-0.98]	0.01	0.94	1.13	3.13	0.96 [0.93-0.98]	0.16	0.35
	Asymptomatic (N=37)	1.15	3.20	0.97 [0.95-0.99]	0.18	0.25	1.04	2.89	0.94 [0.89-0.97]	0.23	0.17
Examiner 2	All participants (N=74)	1.29	3.57	0.93 [0.91-0.96]	0.24	0.04	1.22	3.37	0.91 [0.87-0.94]	0.23	0.05
	Symptomatic (N=37)	1.23	3.42	0.96 [0.93-0.98]	0.13	0.45	1.28	3.56	0.91 [0.86-0.95]	0.07	0.69
	Asymptomatic (N=37)	1.34	3.71	0.89 [0.81-0.93]	0.37	0.02	1.15	3.17	0.90 [0.83-0.94]	0.25	0.13

Mean MG loss in the upper and lower eyelids as measured by Examiner 1 during the first session were significantly positively correlated for all participants ($\rho=0.57$, $p<0.001$), symptomatic participants ($\rho=0.50$, $p= 0.002$) and asymptomatic participants ($\rho=0.60$, $p=0.0003$). The relationship between the OSDI score (i.e., symptoms of dry eye) and mean MG loss for all subgroups was examined using correlation analysis, as show in Table 6.42. Pearson's correlation was applied to the upper eyelids. Spearman correlation was applied for the lower eyelids as the data were not normally distributed. There was no significant association between the OSDI score and mean MG loss for most conditions.

6.5 Discussion:

This study examined the inter-session repeatability, inter- examiner reproducibility and within-subject variability of the Cobra HD fundus camera in participants who were classified according to their dry eye symptoms. The mean MG loss in the upper and lower eyelids showed significantly positive correlations between the first and second sessions and between the two examiners. In addition, no significant difference was found between the repeated measurements, in both sessions and for both examiners, indicating good inter-session repeatability and inter-examiner reproducibility. The within subject variability of the measurements for both examiners, both eyelids and sessions had Sw values of ≤ 1.34% and ICC values ≥ 0.89 for all subgroups, indicating low within subject variability.

Clinical significance

For the Cobra HD fundus camera, MG loss is classified using the meiboscale developed by Pult and Nichols, [75] and clinical treatment is based on the meiboscale grading. [80] As noted earlier, the step size between levels in this grading scale is approximately 25% [75]. Therefore, if there are differences between examiners or between sessions that are equivalent to 25% or greater, these are considered clinically significant. In the present investigation, differences that were half that step size, i.e., 12.5%, were considered clinically insignificant.

The mean MG loss in the inter-session repeatability and inter-examiner reproducibility experiments for all participants as well as the symptomatic and asymptomatic subgroups was greater in the upper than the lower eyelid. This finding is similar to AlDarrab et al. [71], who reported MG loss of 0.22±0.54% in the upper versus 0.14±0.35%, in the lower eyelids. This finding is also similar to Golebiowski et al. [72] who reported MGD scores of 7.3±6.2 and 2.0±2.8 in the upper and lower eyelids, respectively. However, it differs from the findings of Garduño et al. [7] who compared MG loss quantified using the Cobra HD vs. Antares devices (CSO, Florence, Italy) in 80 participants with (N=26) and without evaporative dry eye (N=54), based on the TFOS DEWS II classification criteria. They reported higher MG loss in the lower compared with the upper eyelids for all subgroups except the evaporative dry eye subgroup. These discrepant findings may be due to differences in the age of the cohorts in the two studies. The mean age in the present investigation was 23 years, ranging between 19-43 years, whereas the Garduño et al. [7] study cohort had a mean age of 37 years, ranging between 18-78 years. Differences in methodology could also account for the discrepant findings. In the present investigation, lid eversion was achieved manually. Conversely, as described in their discussion, Garduño et al. [7] used a device to evert the eyelids. Thus, the varying techniques may expose different areas of the eyelids leading to discrepancies in the measurements. Inter-examiner reproducibility of mean MG loss was better for the upper than the lower eyelid, as the correlation and ICC values were higher (Table 1.6). This is in accordance with Dogan et al. [11] who reported moderate to good agreement between examiners for the upper eyelid and fair to moderate agreement for

the lower eyelid using the Sirius (CSO, csoitalia.it) corneal topographer on 30 outpatient clinic subjects. Differences in reproducibility values of the MG loss from the upper versus lower eyelids may be due to the morphological diversity of the two eyelids. [38] Although lower eyelid evaluation appears to be more practical because of the ease of eversion, the excessive area over the tarsus can be erroneously marked due to the laxity of the lower eyelid in the free-hand tool. [11]

Despite the morphological variations of MGs in the upper and lower eyelids, the present study found, similarly to Pult et al. [74], that the MG loss in the upper and lower eyelids of all experimental sub-groups showed significant positive correlation. Furthermore, MG loss did not vary significantly between symptomatic and asymptomatic sub-groups, in either eyelid, for both examiners.

In the present study, did not find an association between OSDI scores and mean MG loss for most experimental conditions. This finding is consistent with other studies that did not find a correlation between OSDI scores and dry eye signs. [11], [88] For example, Machalińska et al. [89] analysed the association between MG characteristics and tear film-related factors and found that OSDI scores did not correlate with functional and morphological MG parameters in contact lens wearers. Additionally, Dogan et al. [11] found no correlation between OSDI scores and MG loss rate, for both the upper and lower eyelids, while investigating the inter-examiner reliability of meibography evaluation and the impact of eyelid selection for the procedure. Adil et al. [88] investigated the relationship between MG morphology and clinical dry eye tests in patients with MGD, and found no correlation between OSDI and any MG morphological parameter. However, it should be noted that the OSDI questionnaire does not differentiate aqueous deficiency from evaporative dry eye disease, which is most commonly caused by MGD. [89] Additionally, Adil et al. [88] suggested that MG loss is a morphological sign that reflects early stages of MGD, which precedes symptoms.

The findings of of this study are comparable with previous studies of MGD using the Phoenix software for image analysis which is included in the Cobra HD fundus camera, the Antares topographer and the Sirius Scheimpflug camera topographer. [90] Dogan et al. [11] examined the inter-

examiner reproducibility between three examiners using the Sirius corneal topographic device. They observed mean differences of 4% in the upper eyelids and 1% in the lower eyelids with ICC values of 0.87 and 0.85 for the upper and lower eyelids, respectively. Thus, they concluded that the Sirius corneal topographic device provides moderate to good agreement of MG loss of the upper eyelids and fair to moderate agreement of MG loss of the lower eyelids.

Garza-Leon et al. [9] evaluated the agreement between two different software tools, i.e., Phoenix and ImageJ using the Antares meibographer, each analysed by two different examiners. In their investigation, only one set of photographs was collected from each participant, and was subsequently analysed offline by two separate examiners. While not their primary study goal, they reported a within subject variability (ICC) of 0.99. They also reported a mean difference between observers when assessing the same image using the Phoenix program of 0.45% MG loss. In the Garza-Leon et al. [9] study, two examiners assessed one set of images from each participant. In contrast, in the present study, each examiner captured and analysed their own images of the subjects. These methods more closely resemble the clinical setting in which examiners both capture and analyse the images. The mean difference between the MG loss assessed by the two examiners for all participants was -0.37±3.20% and -0.42±2.29% for the upper and lower eyelids, respectively. This is similar to the mean difference between two examiners assessing the same image (0.45%) reported by Garza-Leon et al. [9]. Thus, our findings indicate that the repeatability of the Cobra HD instrument is similar to other meibographers evaluating MG loss using the Phoenix software.

One of the limitations of the study may be that the study included only healthy participants, up to 45 years old that were non-CL wearers and the results may be different in diseased populations or in older populations.

Although the Cobra HD fundus camera meibographer has been previously compared with the Antares instrument and they were found to be interchangeable, [7] the Cobra HD device has not been compared with other commonly used meibographers such as the OCULUS Keratograph. Future studies should evaluate the interchangeability of these instruments.

Conclusions:

In conclusion, the present study examined the inter-examiner reproducibility, inter-session repeatability and within-subject variability of MG loss quantified using the Cobra HD fundus camera meibographer. Differences between the examiners and sessions were < 12.5%, which is the half of the minimum step size when discriminating between grades on the meiboscale. [75] Thus, the Cobra HD fundus camera meibographer demonstrates good repeatability and reproducibility, and clinically similar findings should be obtained when used by different examiners on different occasions. Thus, it is suitable for meibographic assessment and follow-up of disease progression or treatment outcomes.

7. Study 3: Contact Lens Wear and After-Care and its Association with Signs and Symptoms of Meibomian Gland Dysfunction

Submitted for publication, April 2023

7.1. Methods:

The study complied with the ethical principles of the Declaration of Helsinki and commenced after approval from the internal ethics committee of Hadassah Academic College. The methods were explained verbally and participants signed a statement of informed consent prior to their participation.

7.11 Subjects:

In this prospective study, 128 participants between the ages of 18-39 were recruited from the clinics at the Department of Optometry (HAC, Jerusalem, Israel), and from advertisement posted in HAC and social media.

The participants were divided into three cohorts; FLU (followed-up) CL wearers, non-FLU (non followed-up) CL wearers and self-reported “healthy” controls who had never worn CLs.

All CL wearers wore soft CL wearers of all modalities, at least five days a week and at least five hours a day, with at least one year of CL use. [23]

Fitted CL wear was considered CL wearers that had undergone a CL follow up assessment by an eye care practitioner at a least once during the past year. This group was subsequently referred to as FLU (followed-up). The rationale for this criterion is that the time interval between routine professional aftercare visits for soft CLs is 12 months. [91] Over the counter CL wear was defines as CL wear without attending follow-up assessment visits by ab eye care practitioner for at least a year. Thus, this group was subsequently referred to as non-FLU (non followed-up). The control group comprised healthy participants who had never worn CLs.

Exclusion criteria for all groups included ocular infection, inflammation, allergy, past ocular surgery, ocular diseases such as keratoconus, systematic

diseases (e.g., hypertension, diabetes mellitus, ischemic heart disease), or use of medications that might affect the tear film (such as antihistamines and hormones). Previous or current RGP lens wearers, pregnant or lactating women were also excluded.

Inclusion and exclusion criteria were determined based on participants' responses to a systemic medical and ocular history questionnaire that was adopted from Machalińska et al. [23]

Participants arrived wearing their habitual CL for at least one hour prior to the study visit.

7.12 Experimental procedures:

All examinations took place in the contact lens clinic at the Department of Optometry at HAC, in a designated examination room. The monocular distance LogMAR visual acuity was measured with the participant's habitual correction, including a subjective over-refraction by a licensed optometrist (RI) if needed. Corneal topography (Sirius Scheimpflug Camera, Bon Optic VertriebsgmnH, Germany) was performed in order to preclude keratoconus, which is a risk factor for MGD. [64], [65] CL wearers also filled out a questionnaire pertaining to their CL modality, replacement schedule, solutions, habits of wear and hygiene. [92] Extended-wear was defined as napping or sleeping with CLs. Both eyes of each participant were evaluated and only the right eye was included in the analysis.

Morphological measurements

Morphological outcomes included MG loss assessed by meibography and lid margin abnormalities (LAS) of the upper and lower eyelids.

Meibography was measured using the non-contact Cobra HD non-mydriatic digital fundus camera meibographer (CSO and bon Optic VerttiebsgmbH, Italy), which has been shown to have good repeatability and reproducibility. [93] The Cobra HD meibographer requires that the examiner marks the area of the upper and lower eyelids, as well as the area in which the MGs are observed. The software subsequently calculates the percentage and the degree of MG loss (0-100%, 0-4) where 0 represents no MG loss; 1 represents less than 25% loss, 2 represents 26–50% loss, 3 represents 51–

75% loss and 4 represents >75% MG loss. [10] A meiboscore ≥2 is considered abnormal. [41]

Slit-lamp biomicroscopy (Huvitz Slit Lamp HS-5000) was performed to assess seven lid margin abnormalities (LAS) including narrowed MG orifices, plugged MG orifices, posterior displacement of the orifices, lid margin telangiectasia, posterior lid margin hyperemia, rounding of the posterior margin, and notching of the lid margin, in the upper and lower eyelids, which were scored as either grade 0 (absent) or grade 1 (present). [23] The sum of the total lid margin abnormalities provided a combined score, with 0 signifying no abnormalities present and 7 signifying all abnormalities present. [23]

Functional measurements

Functional outcomes included meibum quality and expressibility, invasive and non-invasive tear break up time, Schirmer test, corneal and conjunctival staining and MGD grade of both eyelids. The meibum quality score (MQS) was obtained by applying pressure to the eight central glands in the lower tarsus and maintaining the pressure for 10 seconds. MQS was recorded as follows: grade 0, clear fluid; grade 1, cloudy fluid; grade 2, cloudy, particulate fluid; and grade 3, Similar to a toothpaste. [41] Meibum Expressibility Score (MES) was graded as follows: grade 0, all glands expressible; grade 1, 3 to 4 glands expressible; grade 2, 1 to 2 glands expressible; and grade 3, no glands expressible. [41] In both MQS and MES, a score ≥2 was considered abnormal. [41] Tear break up time (TBUT) was estimated by placing a single fluorescein strip over the inferior tear meniscus after instilling a drop of saline, under cobalt blue illumination. The time from the last blink to the first appearance of dry spot on the cornea was recorded with a stopwatch. The procedure was repeated three consecutive times, and the mean was calculated [23] with TBUT values lower than 10 seconds considered abnormal. [23] The non-invasive tear film break-up time (NITBUT; Sirius Scheimpflug Camera, Bon Optic VertriebsgmnH, Germany) based on the time (in seconds) required for the first distortion to appear on the reflected Placido disc rings, was measured three consecutive times and the mean was calculated. [36] A value of ≤10 sec considered abnormal. [41] Schirmer test (without topical anesthesia) was performed with closed eyes, by placing a

paper strip in the mid-lateral part of the lower fornix. The amount of wetting after five minutes was documented, [23] and the cutoff value was set at <5 mm in 5 minutes [41], [94] Corneal and conjunctival staining were evaluated based on Efron's grading scale, using the slit lamp biomicroscope and scored in binary form as 0 ;no staining was detected and clinical action not required or 1; staining was detected and clinical action is required. [94] MGD grade was determined based on Efron's grading scale (0-4, Figure 7.13), [94] using the slit lamp biomicroscope. Grade 0; Normal: Clinical action not required, Grade 1; Trace: Clinical action rarely required, Grade 2; Mild: Clinical action may be required, Grade 3; Moderate: Clinical action usually required, Grade 4; severe: Clinical action certainly required.

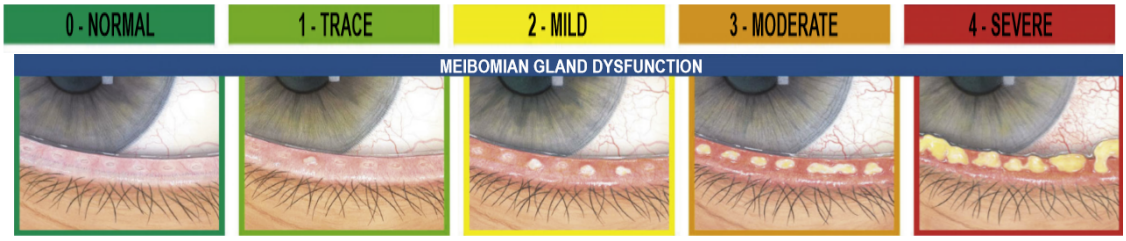


Figure 7.13: MGD assessment according Efron's grading scale
MGD grade can determined based on Efron's grading scale (0-4)

Subjective measurements

Subjective outcomes were assessed with the Ocular Surface Disease Index (OSDI). [95] Because the OSDI questionnaire does not differentiate between aqueous deficiency and evaporative DE disease, [89] also the MGD symptoms questionnaire (based on Machalińska et al. [89]), which includes more specific characteristics of clinical symptoms associated with MGD was also included.

The OSDI score was calculated as follows: (sum of scores for all questions answered) X 25/ (total number of questions answered) and rated on a scale of 0 to 100. [48] Participants with OSDI scores ≥13 were classified as symptomatic. [70]

7.2 Statistical Analysis:

Sample size

Based on prevalence of MGD in the three cohorts as the main study variable, the minimum sample size was calculated based on an assumed average prevalence of MGD in CL wearers of 49% [96] and a prevalence of MGD of 12.5% in non-CL wearers [97] with a 95% confidence level and 80% power. This calculation resulted in a minimum required sample size of 22 participants in each group for statistically significant difference. [98]

Analysis of Study Outcomes

Demographic data was evaluated using descriptive statistics. The mean and standard deviation of the percentage of MG loss of the participants' right eyes were calculated.

The normality of the outcome measures was assessed using the Kolmogorov-Smirnov test.

For outcome variables with binary scales, a Pearson Chi-squared test was applied to compare between the cohorts. Outcome variables with a continuous scale (0-4) were compared between cohorts using Mann-Whitney or Kruskal-Wallis tests with post-hoc analysis.

A univariate logistic regression model adjusted for group (control, FLU or non-FLU) was used to examine whether wearing CLs is an independent risk factor of certain morphologic or functional abnormality in the MGs or specific ocular symptoms.

The CL wearer data were analyzed both as a single cohort (CL wearers) as well as two separate cohorts; FLU CL wearers, non-FLU CL wearers.

Statistical analysis was performed with Microsoft Office Excel (Microsoft Corporation, Redmond, WA, USA) and IBM SPSS Statistics (version.27, IBM Corporation, USA). P-values lower than 0.05 were considered significant.

7.3 Results:

7.31 Subjects:

The right eyes of 128 healthy participants, 95 (74.2%) females, aged 18-39 years (mean age: 22.5±3.8 years) were included in this experiment: Followed-Up CL wearers (N=31, 24.2%), non-Followed Up CL wearers (N=43, 33.6%) and control non-CL wearers (N=54, 42.2%).

The demographic data for the three cohorts are detailed in Table 7.32. A chi-square test of independence showed a significant difference between the groups in the number of females ($X^{2(2)}$= 8.66, p= 0.01). Post hoc (Bonferroni) analysis revealed significantly more females in the non-FLU group compared to the control group. There were no significant age differences between the cohorts (Kruskal-Wallis test, p=0.90, Table 7.32).
The CL lens modality of all CL wearers is detailed in Table 7.33. There was no significant difference in the CL modality between the Fitted and non-FLU cohorts ($X^{2(1)}$= 0.88, p= 0.64, Table 7.33).

CL Compliance

CL usage habits and compliance are detailed in Table 7.34. Extended wear refers to the sum of the number of participants that reported either napping or sleeping with their CLs. There was no significant difference between FLU and non-FLU CL wearers in number of years of CL wear, and daily hours of CL wear between the FLU and non-FLU cohorts (p= 0.30, p=0.40, respectively, Table 7.34). Thus, the FLU and non-FLU cohorts were compared using a chi-square test of independence, which showed no significant between the cohorts ($X^{2(1)}$= 0.02, p= 0.90, Table 7.34).

Table 7.32: Demographic data
Demographic data (number of participants, number of female participants, and age) of the three study cohorts; controls (first column), FLU (second column) and non-FLU (third column). The fourth column lists the p values of the chi-square test of independence applied to compare the number of female

participants in the cohorts, and the Kruskal-Wallis test which compared the ages of the cohorts.

		Controls	CL wearers		P value
			FLU	non-FLU	
Participants (% of N=128)		54	31	43	
Female		33 (61.1%)	25 (80.6%)	37(86.0%)	0.01
Age (years)	**Mean ± SD**	22.3±3.5	22.2±3.1	23.0±4.6	0.90
	Range	18-39	18-33	18-37	

Table 7.33: CL modality of CL wearers

The distribution of CL types used by the FLU and non-FLU cohorts in this study. The X^2 statistic showed no significant difference between the CL types used by the FLU and non-FLU cohorts.

	FLU **(N=31)**	non-FLU **(N=43)**	**P value**
Daily	10 (32.3%)	10 (23.3%)	0.64
Bi-weekly	3 (9.7%)	6 (13.9%)	
Monthly	18 (58.1%)	27 (62.8%)	

Table 7.34: CL usage habits and compliance in CL wearers
The CL usage habits and compliance of the FLU (first column) and non-FLU (second column) cohorts, with the p-values of the Kruskal-Wallis test that was employed to compare between the cohorts (third column). Extended wear refers to the number of participants that reported napping or sleeping with their CLs. This binary variable was compared using the X^2 statistic.

	FLU **(N=31)**	non-FLU **(N=43)**	**P value**
Years of CL wear	5.13±3.17	6.56±4.96	0.30
Daily hours of CL wear	10.90±2.91	11.67±2.91	0.40
Extended wear	12 (38.7%)	16 (37.2%)	0.90

The distance visual acuity, refraction, over-refraction, and keratometry (K) readings outcome measures are tabulated in Table 7.35. There was a significant difference in the distance VA (approximately 0.1 LogMAR, p=0.002) and for the refractive spherical component (approximately 2.5D, p=0.00) between CL wearers and non-CL wearers. There was no significant difference between their refractive cylindrical component (p=0.30), over-refraction (p=0.79) and K-readings (p=0.25).

When analysed as three cohorts, there was a significant difference between in the distance VA (approximately 0.07 LogMAR, p=0.008) and the refractive spherical component (approximately 2.5D, P<0.00001). Post-hoc comparisons (Bonferroni) found that the controls had significant better VA than the non-FLU CL wearers (p=0.002), with no significant differences between other groups. The FLU and non-FLU cohorts had a significantly higher refractive spherical component compared with the non-CL wearing control cohort (both p<0.000), without significant differences between the FLU and non-FLU cohorts (p=0.89). The cohorts did not differ significantly in their refractive cylindrical component (p=0.37), over-refraction (p=0.48) and K-readings (p=0.13).

Table 7.35: Distance Visual Acuity, Refraction, and Keratometry outcome measures

Distance VA, spherical and cylindrical refractive components, over-refraction (OR) and K-readings of the experimental cohorts. The column with P-values includes two values, the upper representing the results of Mann-Whitney U tests comparing between two cohorts: CL wearers vs. Non-CL wearing controls; and the lower representing the results of the Kruskal-Wallis tests comparing between three cohorts: FLU, non-FLU and control group.

Test	Controls (N=54)		CL wearers (N=74)		CL wearers				P value
					FLU (N=31)		non-FLU (N=43)		
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Distance VA (LogMAR)	0.93± 0.12	0.63-1.0	0.84± 0.16	0.32-1.0	0.89± 0.14	0.5-1.0	0.82± 0.17	0.32-1.0	0.002 0.008
Sph (D)			-3.49±	-11.50-	-3.38±	-7.00-	-3.52±	-11.50-	0.00

	-0.82± 1.74	-7.00- +1.50	2.26	+4.75	2.17	+4.75	2.36	+3.00	<0.00001
Cyl (D)	-0.24± 0.47	-2.50- 0	-0.22± 0.51	-2.25- 0	-0.31± 0.58	-2.25- 0	-0.16± 0.46	-1.75- 0	0.30
									0.37
OR (D)	-0.09± 0.21	-0.75- +0.5	-0.07± 0.13	-0.5- 0	-0.04± 0.11	-0.5- 0	-0.08± 0.13	-0.5- 0	0.79
									0.48
K readings (mm)	7.77± 0.34	7.12- 8.67	7.71± 0.26	6.84- 8.25	7.65± 0.32	6.84- 8.25	7.76± 0.21	7.34- 8.24	0.25
									0.13

Morphological outcome measures

Morphological outcome measures are tabulated in Table 7.36. CL wearers differed significantly from control non-CL wearers in the mean MG loss of the upper eyelids (p=0.00) and in lid margin abnormalities of the lower eyelids (p=0.03). They did not differ in the mean MG loss in the lower eyelids (p=0.67) and in lid margin abnormalities of the upper eyelids (p=0.09).

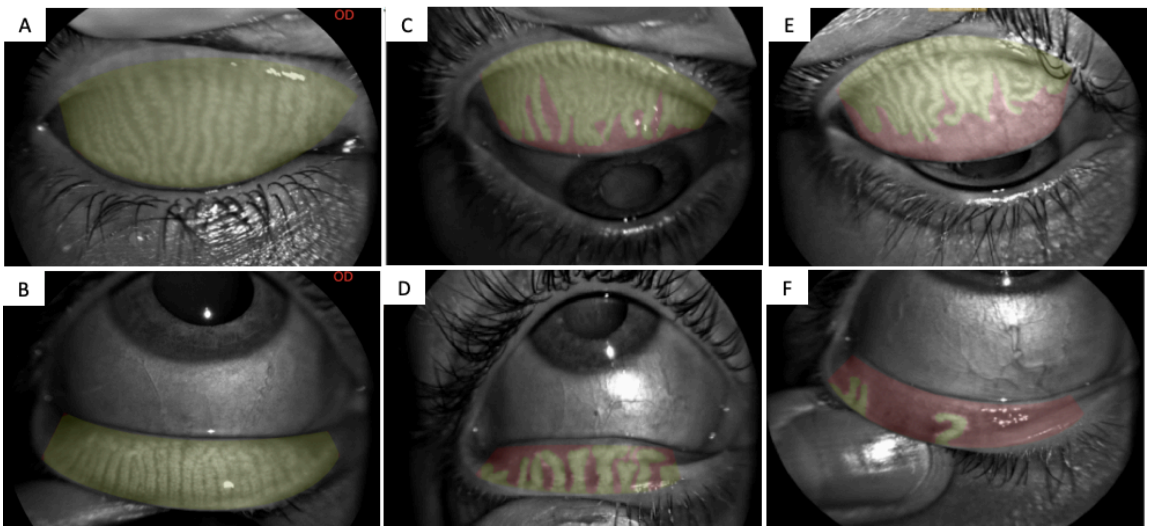
When analyzed as three cohorts they differed significantly in the mean MG loss in the upper eyelids (p=0.0004). Post-hoc comparisons (Bonferroni) indicated that the mean MG loss of the upper eyelids was significantly higher in CL wearers (both cohorts) compared with non-CL wearing controls (FLU: p=0.02, non-FLU: p=0.001, respectively) but not between the FLU and non-FLU CL wearers (p=0.58). The cohorts did not differ in the mean MG loss in the lower eyelids (p=0.74) and in lid margin abnormalities of both eyelids (upper eyelids: p=0.32, lower eyelids: p=0.09). Representative Cobra HD meibography images of the upper and lower eyelids obtained from a non-CL wearer, a FLU CL wearer, and a non-FLU CL wearer by can be seen in Figure 7.37.

Table 7.36: Morphological outcome measures

The mean MG loss (in percentages) and lid margin abnormalities (LAS, scored on a scale of 0-7) of the experimental cohorts. The column with P-values includes two values, the upper representing the results of Mann-Whitney U tests comparing between two cohorts: CL wearers vs. non-CL wearing controls; and the lower representing the results of the Kruskal-Wallis tests comparing between three cohorts; FLU, non-FLU and control.

Test		Controls (N=54)		CL wearers (N=74)		CL wearers				P value
						FLU (N=31)		non-FLU (N=43)		
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Mean MG loss (%)	Upper lid	11.2± 6.8	0.3-28.8	17.9± 10.3	2.5-67.1	16.9± 8.8	4.5-38.8	18.6± 11.3	2.5-67.1	0.00
										0.0004
	Lower lid	8.0± 8.48	0.3-41.1	9.7± 12.4	0.1-87.6	7.9± 8.5	0.3-40.5	11.0± 14.5	0.1-87.6	0.67
										0.74
LAS	Upper lid	0.3± 0.5	0-2	0.6± 1.0	0-4	0.6± 1.0	0-4	0.7± 1.0	0-3	0.09
										0.32
	Lower lid	1.0± 1.0	0-3	1.5± 1.4	0-6	1.2± 1.2	0-4	1.7± 1.6	0-6	0.03
										0.09

Figure 7.37: Representative Cora HD meibography images of the upper and lower eyelids obtained from a control non-CL wearer, a FLU CL wearer, and non-FLU CL wearer.
(A+B) Upper and lower eyelids of 23 years old female, non CL wearer. MG loss was not observed in both eyelids (Degree 0, mean MG loss 0%). (C+D) Upper and lower eyelids of 22 years old female, FLU CL wearer. MG loss was observed in the upper (Degree 1, MG loss 19.4%) and the lower eyelids (Degree 2, MG loss 42.2%). (E+F) Upper and lower eyelids of 18 years old male, non-FLU CL wearer. MG loss was observed in the upper (Degree 2, MG loss 34.7%) and the lower eyelids (Degree 4, MG loss 86.5%).



Functional outcome measures

Functional outcome measures are shown in Table 7.38. CL wearers differed significantly from control non-CL wearers in the meibum expressibility score (p=0.008), and not in other functional outcome measures.

When analyzed as three cohorts, they differed significantly in the meibum expressibility score (p=0.004), with post-hoc comparisons (Bonferroni) demonstrating that non-CL wearers had significantly lower meibum expressibility scores compared to FLU (p=0.01) and non-FLU (p=0.0008) CL wearers. No statistically significant differences between the groups was observed for the other functional outcome measures.

Correlation between MGD grades determined by different techniques

The functional MGD grade (0-4) determined according Efron grading scale was not significantly correlated to the morphological MGD grade (0-4) determined according to the Cobra HD meiboscale (upper eyelid: Rho 0.03, p=0.70, lower eyelid: Rho 0.11, p=0.23).

Table 7.38: Functional outcome measures

Meibum quality score, meibum expressibility score, TBUT, NITBUT, Schirmer test and MGD grade according Efron grading scale in the upper and lower eyelids for the three cohorts is listed. The column with P-values includes two values, the upper representing the results of Mann-Whitney U tests comparing between two cohorts; and the lower representing results of the Kruskal-Wallis tests comparing between three cohorts; FLU, non-FLU and control.

Test	Control (N=54)		CL wearers (N=74)		CL wearers				P value
					FLU (N=31)		non-FLU (N=43)		
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Meibum Quality score (0-3)	0.3± 0.6	0-2	0.3± 0.6	0-3	0.1± 0.3	0-1	0.4± 0.8	0-3	0.28 0.43

Meibum Expressibility score (0-3)		0.2± 0.5	0-3	0.7± 0.8	0-3	0.6± 0.7	0-2	0.8± 0.9	0-3	0.008 0.004
TBUT (sec)		8.3± 4.6	3.7-24.7	9.1± 4.8	3.3-23.1	9.3± 5.0	3.7-23.1	8.9± 4.6	3.3-21.6	0.35 0.62
NITBUT (sec)		10.0± 4.6	3.2-17.4	9.9± 4.5	1.4-21.0	9.4± 4.2	3.7-21.0	10.2± 4.7	1.4-21.0	0.93 0.76
Schirmer test (mm)		11.8± 7.1	0-35	11.6± 6.6	2-35	13.2± 7.3	2-35	10.4± 5.9	2-35	0.51 0.18
MGD grade: Efron (0-4)	**Upper lid**	0.2± 0.6	0-2	0.3± 0.6	0-2	0.1± 0.3	0-1	0.4± 0.7	0-2	0.23 0.23
	Lower lid	0.4± 0.9	0-4	0.5± 0.7	0-3	0.5± 0.8	0-3	0.6± 0.7	0-2	0.06 0.22

Subjective outcome measures

The subjective outcome measures are shown in Table 7.39. The MGD symptoms questionnaire score of the CL wearers was significantly higher than the control non-CL wearers (p=0.008), while the OSDI score did not (p=0.75). When analyzed as three cohorts, they differed significantly in the MGD symptoms questionnaire score (p=0.02), with post-hoc comparisons (Bonferroni) showing that non-FLU had significantly higher ocular symptoms compared with the non-CL wearing controls (p=0.002). The symptoms score of the FLU non-FLU vs. non-FLU cohorts approached significance (p=0.05). No significant difference in the OSDI score emerged between the cohorts (p=0.18).

Table 7.39: Subjective outcome measures

Subjective outcome measures (OSDI score and ocular symptoms questionnaire score) of the cohorts. The column with P-values includes two values. The upper value represents results of Mann-Whitney U tests comparing between two cohorts: CL wearers vs. non-CL wearing controls. The lower value represents results of Kruskal-Wallis tests comparing between three cohorts; FLU, non-FLU and control group.

Test	Control (N=54)		CL wearers (N=74)		CL wearers				P value
					FLU (N=31)		non-FLU (N=31)		
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
OSDI Score (0-100)	17.1± 17.0	0-77	15.6± 15.0	0-60.4	11.6± 11.4	0-50	18.4± 16.6	0- 60.4	0.75 0.18
Ocular symptoms questionnaire score (0-11)	2.3± 2.1	0-8	3.3± 2.3	0-9	2.7± 2.2	0-8	3.7± 2.4	0-9	0.008 0.02

A univariate logistic regression model (Table 7.4) adjusted for cohort was used to examine if CL wear is an independent risk factor of specific functional or morphological MG abnormalities. When all CL wearers (both FLU and non-FLU) were compared with the control, non-CL wearers, CL use was significantly associated with increased corneal staining (p=0.002), increased conjunctival staining (p=0.0001), MG loss stage ≥2 in the upper eyelid (p=0.01) and lid margin telangiectasia (p=0.01).

When analyzed as three cohorts, non-FLU CL wear was found to be significantly associated with abnormal meibum quality score (stage ≥2) (p=0.04), increased corneal staining (p=0.001), increased conjunctival staining (p=0.0005), MG loss stage ≥2 in the upper eyelid (p=0.01) and lid margin telangiectasia (p=0.003). FLU CL wear was associated with increased corneal staining (p=0.001) and MG loss stage ≥2 in the upper eyelid (p=0.04).

Table 7.4: Incidence of specific MGD signs in CL wearers and non-CL wearers

Results of the univariate logistic regression model adjusted for cohort that examined if CL wear is an independent risk factor of specific functional or morphological MG abnormalities.

Parameters	Control (N=54)	CL wearers (N=74)	FLU (N=31)	non-FLU (N=43)	Comparison	OR	95% CI	P
Meibum quality score; stage ≥2	1.9%	5.4%	0%	9.3%	CL wearers - Control	5.47	0.53-56.09	0.15
					FLU-Control	0.00	0.00-0.00	1.00
					non-FLU-Control	12.87	1.12-148.41	0.04
Meibum expressibility score; stage ≥2	9.3%	18.9%	12.9%	23.3%	CL wearers - Control	2.45	0.79-7.59	0.12
					FLU-Control	1.56	0.37-6.45	0.54
					non-FLU-Control	3.25	0.97-10.93	0.06
TBUT ≤10 sec	81.5%	73%	71%	74.4%	CL wearers - Control	0.44	0.17-1.14	0.09
					FLU-Control	0.41	0.14-1.25	0.12
					non-FLU-Control	0.47	0.16-1.35	0.16
NITBUT ≤10 sec	50%	54.8%	61.3%	48.8%	CL wearers - Control	0.88	0.41-1.89	0.75
					FLU-Control	1.29	0.49-3.36	0.60
					non-FLU-Control	0.68	0.29-1.60	0.37
Schirmer test < 5 mm in 5 min	18.5%	8.1%	3.2%	11.6%	CL wearers - Control	0.33	0.11-1.01	0.05
					FLU-Control	0.13	0.01-1.06	0.06
					non-FLU-Control	0.49	0.15-1.61	0.24
Corneal staining; 0/1	16.7%	40.5%	35.5%	44.2%	CL wearers - Control	4.35	1.72-11.00	0.002
					FLU-Control	3.42	1.16-10.11	0.03
					non-FLU-Control	5.23	1.89-14.48	0.001
Conjunctival staining; 0/1	7.4%	39.2%	22.6%	51.2%	CL wearers - Control	7.45	2.39-23.24	0.001
					FLU-Control	3.44	0.90-13.07	0.07
					non-FLU-Control	12.18	3.66-40.51	0.00005
MG loss-upper eyelid; stage ≥2	1.9%	20.3%	16.1%	23.3%	CL wearers - Control	13.78	1.71-110.93	0.01
					FLU-Control	10.47	1.14-96.29	0.04

					non-FLU-Control	16.63	1.96-140.86	0.01
Narrowed MG orifices; 0/1	1.9%	5.4%	6.5%	4.7%	CL wearers - Control	4.05	0.40-40.73	0.23
					FLU-Control	4.67	0.38-57.36	0.23
					non-FLU-Control	3.54	0.28-44.56	0.33
Posterior displacement of the orifices; 0/1	9.3%	13.5%	9.7%	16.3%	CL wearers - Control	2.55	0.73-8.96	0.14
					FLU-Control	1.60	0.33-7.90	0.56
					non-FLU-Control	3.54	0.88-14.20	0.07
Lid margin telangiectasia; 0/1	25.9%	48.6%	38.7%	55.8%	CL wearers - Control	2.50	1.27-6.17	0.01
					FLU-Control	1.87	0.71-4.90	0.20
					non-FLU-Control	3.78	1.55-9.21	0.003
Rounding of the posterior lid margin; 0/1	11.1%	17.6%	12.9%	20.9%	CL wearers - Control	2.37	0.77-7.29	0.13
					FLU-Control	1.58	0.38-6.44	0.53
					non-FLU-Control	3.14	0.92-10.74	0.07
Notching of the lid margin; 0/1	9.3%	8.1%	6.5%	9.3%	CL wearers - Control	0.87	0.24-3.16	0.84
					FLU-Control	0.68	0.12-3.85	0.67
					non-FLU-Control	1.02	0.24-4.26	0.98

7.5 Discussion:

This investigation examined the relationship between CL wear and morphological, functional and subjective symptoms of MGD. Unlike past studies [20], [24], [26] examining this relationship, the present investigation used the International Workshop on MGD definition of MGD.[1] The present investigation also included a control, non-CL wearing cohort. It also included an objective an automatic instrument for assessing MGD. Due to a unique situation in Israel that allows the consumers to obtain CLs without requiring a prescription or follow-up, this investigation is also unique in that the results of the study could compare between FLU and non-FLU CL wearers. Significantly more morphological and functional MG changes were found in all CL wearers

compared with the control, non-CL wearers. Further, the univariate logistic regression analysis demonstrated that non-FLU soft CL wear is an independent predictor of more functional and morphological abnormalities compared with FLU CL wear.

Non-FLU CL wearers also had significantly reduced VA compared with the control cohort. This finding can be explained by the fact that the CLs were obtained either obtained by the user without an initial fitting which would have included an examination, or continuous wear of CLs without follow-ups, which would have included a refractive examination. It is possible that the reduced VA and increased MG abnormalities associated with CL wear may be due to improper fit or after-care. This hypothesis is supported by Young et al. [37] who found several factors associated with unregulated supply of CLs that may lead to an increased risk of complications. First, the CLs are provided without any verification that the lenses give an acceptable fit. Those that purchase CLs from unregulated suppliers do not undergo training in CL hygiene, which is considered an essential part of the fitting process. Third, there is a probability of delay in seeking professional help in case of problems, without the supervision of CL practitioner.

Machalińska et al. [23] also showed, using a multivariate logistic regression analysis, that CL use is associated with abnormal meibum quality, more frequent bulbar and palpebral conjunctival hyperemia, lid margin telangiectasia, rounding, notching, hyperemia of the posterior lid margin, orifice plugging, and retroplacement. Thus, the observed abnormalities may be a consequence of CL wear.

The mean MG loss in the upper eyelids was significantly higher in both FLU and non-FLU CL wearers compared with non-CL wearing controls ($p=0.02$, $p=0.001$, respectively), which is consistent with several previous studies. [8], [17]–[19] Alghamdi et al. [18] observed the characteristics of the MGs, eyelids and tear film following short (2±1 years), moderate (5±1 years) and long (10±2 years) duration of soft CL wear. They concluded that MG loss in both eyelids was significantly higher in CL wearers of all duration profiles compared with non-CL wearers ($p=0.001$). Arita et al. [19] investigated the influence of CL wear on the MGs of the upper and lower eyelids in soft and

rigid CL wearers and reported that CL wearers have significantly less MGs in both eyelids compared with non CL-wearers (upper eyelid; $p<0.0001$, lower eyelid; $p=0.036$), with greater MG loss in the upper eyelid compared with the lower eyelid. They showed that the shortening of the MGs in CL wearers began from the distal side in both eyelids suggesting that chronic irritation of MGs by CLs was a major causative mechanism for MG changes in CL wearers. Harbiyeli et al. [17] evaluated the structural changes in MGs of soft and rigid CL wearers compared with control group. They found that the mean MG loss was 25.3±12.5% in soft CL wearers, 34.0±13.4% in rigid CL wearers, and 18.4±9.2% in the control group ($p<0.001$). The mean MG loss in the upper eyelid was higher in soft CL wearers compared with the control group ($p=0.045$). García-Marqués et al. [8] assessed the effects of soft CL wear and the wear-duration on the tear film, MG loss of the upper eyelids, and MG appearance in the upper eyelids. They showed that CL wearers have significantly more MG loss in the upper eyelid compared with non-CL wearing controls ($p<0.001$). Thus, CL use may predispose individuals to MG loss and this effect may be more pronounced in the upper eyelid. Although the CLs attach to both eyelids, the upper may experience more irritation because it completes larger movements while blinking. [19]

Consistent with previous reports, [15], [18], [28] in this study, the meibum expressibility score was significantly lower in the control non-CL wearing cohort, compared with both the FLU ($p=0.01$) and non-FLU ($p=0.0008$) cohorts, suggesting that soft CL wear leads to reduced MG expressibility. Alghamdi et al. [18] found that non-CL wearers demonstrated significantly higher expressibility compared with soft CL wearers with 1-10 years of CL usage, regardless of the duration of CL use ($p<0.001$). Similarly, Uçakhan and Arslanturk-Eren [15] reported significantly reduced MG expressibility in soft CL wearers (1.3±0.7) compared with non-CL wearing controls (0.8±0.6, $p<0.001$) and Villani et al. [28] also found that soft CL wearers (1.9±0.9) had significantly reduced MG expressibility (0.4±0.6, $p<0.001$).

Non-FLU CL wearers had significantly higher DE symptoms compared with the control non-CL wearers ($p=0.002$). This finding is in accordance with Guillon and Maissa [99] who reported that CL wearers experienced more DE

symptoms compared with non-CL wearers, with dryness being the most prominent symptom amongst soft CL wearers. Despite our findings regarding ocular symptoms, there were no significant differences between the cohorts in the OSDI questionnaire scores. One possible explanation for this discrepancy, is the fact that the OSDI questionnaire asks about symptoms experienced over the past week, whereas the general symptoms questionnaire does not refer to a specific timepoint. Further, the OSDI questionnaire does not distinguish between evaporative dry eye disease and aqueous deficiency, whereas the ocular symptoms questionnaire attempts to identify ocular symptoms associated with MGD. [89] Similarly to our findings, also other past studies found no significant differences in OSDI questionnaire scores between CL wearers and non-CL wearers. [23], [36]

The present study found no significant differences between FLU CL wearers, non-FLU CL wearers and the non-CL wearing controls in the lid margin abnormalities score, meibum quality score, TBUT, NITBUT, MGD score according Efron's scale, and Schirmer test results. Thus the morphological changes observed in MGs with meibography had no detectable effects on tear film and MG function in soft CL wearers. Machalinska et al. [23] reported that meibum quality starts to decrease after about four years of CL use, and that lid margin abnormalities are increased after 10 years of CL wear. In the present study, the duration of CL use was less than 10 years in more than half of the participants (66/85 participants, 77.6 %). However, early morphological changes in MGs that were not accompanied by significant functional changes and lid margin abnormalities were found. MG loss is a morphological sign which is considered an early stage of MGD. [88]

One possible limitation of this study is the over-representation of females in the CL wearing cohorts. Given that MGD is more common in men, [100] the fact that so many signs and symptoms of MGD were found in CL wearers in the present study, coupled with the point that the majority of the participants were female, emphasizes the severity of the effect of CLs on the MGs.

Conclusions:

This study found that CL wear was significantly associated to several alterations in MG morphology and function, including reduced meibum expressibility, corneal staining and upper eyelid MG loss. Non-FLU wear was also significantly associated with DE symptoms, abnormal meibum quality, conjunctival staining and lid margin telangiectasia. These findings stress the importance of CL after-care.

8. General conclusions

1. The topical review conducted on research studies published between 1980-2021 showed that discrepancies between the 15 papers that demonstrated a relationship between CL wear and MGD vs. the seven papers that did not report a relationship are due to several factors. Namely, the definition of MGD, the questionnaires used to assess symptoms, the methodology employed to examine MG dropout and the inclusion of both lids, and the type of CLs. This relationship was prospectively examined in the present dissertation on three cohorts (FLU CL wearers, non-FLU CL wearers, and non-CL wearing controls) using two validated questionnaires and two objective methods of assessing MGs.
2. A study was carried out to examine the MG function of the Cobra HD Fundus camera for inter-session repeatability, inter-examiner reproducibility and within-subject variability on symptomatic and asymptomatic participants. Results showed that the Cobra HD fundus camera meibographer has good repeatability and reproducibility making it suitable for meibographic assessment and follow-up of disease progression or treatment outcomes.
3. The results of the study examining the relationship between CL wear and MGD showed all CL wearers had significantly more morphological and functional MG changes including abnormal meibum quality, more frequent bulbar and palpebral conjunctival hyperemia, lid margin telangiectasia, rounding, notching, hyperemia of the posterior lid margin, orifice plugging, and retroplacement. Non-FLU CL wear was found to be an independent predictor of many functional and morphological abnormalities, more than FLU CL wear. Non-FLU CL wear was also significantly associated with reduced VA. These findings demonstrate that there is a relationship between MGD and CL wear, and that non-FLU CL wear results in more ocular complications compared with fitted CL wear in soft CLs.

9. Future work

To further address the effect of CL wear on MGD, several future studies are suggested:

1. Comparison of ocular complications as well as compliance with handling and usage instructions in non-FLU vs. FLU contact lens wearers vs. controls. In the State of Israel, CLs can be purchased legally over-the-counter. Improper fitting and usage of CLs, however, are known to enhance ocular intolerance and other complications. In my PhD studies I examined MGD in three cohorts of CL wearers. I would like to further investigate CL-related ocular complications by comparing additional parameters of ocular complications between these cohorts.
2. Comparison of the measurement of MG loss in patients with and without dry eye symptoms, between the Cobra HD fundus camera meibographer and Sirius Scheimpflug meibographer using Phoenix software, to determine if they are interchangeable in a clinical setting. There are several devices that allow objective imaging of the MGs. The repeatability and interchangeability of these devices is important for determining the optimal method for diagnosis and management of MGD and if the devices provide similar outcomes.
3. Comparison of signs and symptoms of MGD in patient with keratoconus (KC) that are corrected with contact lenses, patients with KC who are not corrected with contact lenses and non-contact lens wearing controls. KC has been shown to be a risk factor for MGD [64], [65]. Past studies that examined the relationship between MGD and KC excluded CL wearers. Most KC patients require contact lens corrections which provide better visual function. Therefore, examining if the KC patients with typically more advanced MGD have even more MG loss and other signs and symptoms associated with MGD when they wear CLs, may have implications for the management of KC.
4. Comparing the relationship between signs and symptoms of MGD and dyslipidemia in subjects who have high cholesterol values vs. healthy

control participants. Dyslipidemia has been linked to the development of MGD, but direct evidence supporting this relationship is lacking. I would like to explore the link between dyslipidemia and MGD based on techniques developed in this dissertation. [101]

10. Publications and conference presentations

Publications

1. **Ifrah R**, Quevedo L, Gantz L.
   Topical review of the relationship between contact lens wear and meibomian gland dysfunction. *Journal of Optometry*. 2023*;* 16(1):12-19.

2. **Ifrah R,** Quevedo L, Gantz L.
   Repeatability and reproducibility of Cobra HD fundus camera meibography in young adults with and without symptoms of dry eye. *Ophthalmic and Physiological Optics*. 2023**;** 43(2):183-194.

3. **Ifrah R**, Quevedo L, Hazrati G, Maman S, Mangisto H, Shmuel E, Gantz L.
   Contact lens wear and after care and its association with signs and symptoms of meibomian gland dysfunction**.** Submitted to *Ophthalmic and Physiological Optics* in April 2023

Conferences

International Conferences

Dates	Name of Conference	Conference Location	Presentation Title	Role
10.2022	American Academy of Optometry (AAO)	San Diego, USA	MGD and Dry Eye Symptoms of Fitted and Over the Counter Contact Lens Wearers compared with non-Contact Lens Wearing Controls	Poster presentation
05.2022	European Academy of Optometry and Optics (EAOO)	Dublin, Ireland	Validation of the Cobra HD Meibographer	Poster presentation

Conferences in Israel

Dates	Name of Conference	Conference Location	Presentation Title	Role
03.2023	Israeli Society for Vision and Eye Research (ISVER)- an ARVO international chapter affiliate	Technion University, Haifa, Israel	MGD and Dry Eye Symptoms of Fitted and Over the Counter Contact Lens Wearers compared with non-Contact Lens Wearing Controls	Oral presentation
01.2023	Invited lecture	Hadassah Academic College, Jerusalem, Israel	MGD and Dry Eye Symptoms of Fitted and Over the Counter Contact Lens Wearers compared with non-Contact Lens Wearing Controls	Oral presentation
09.2022	Israel Vision Science Society (IVSS)	Bar Ilan University, Ramat Gan, Israel	Repeatability and reproducibility of Cobra HD fundus camera meibography in participants with and without symptoms of dry eye	Poster presentation
03.2022	Israeli Society for Vision and Eye Research (ISVER)- an ARVO international chapter affiliate	Modi'in, Israel	Validation of the Cobra HD Meibographer in Symptomatic and Asymptomatic Patients	Oral & Poster presentation

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