Abstract

Purpose: With the potential to address evaporative dry eye, a novel spray has been developed in which phospholipid liposomes are delivered to the tear film via the surface of the closed eyelid. This study evaluated the short-term effects of liposomal spray application on the lipid and stability characteristics of the pre-ocular tear film in normal eyes.

Methods: Twenty-two subjects (12M, 10F) aged 35.1 ± 7.1 years participated in this prospective, randomised, double-masked investigation in which the liposomal spray was applied to one eye, and an equal volume of saline spray (control) applied to the contralateral eye. Lipid layer grade (LLG), non-invasive tear film stability (NIBUT) and tear meniscus height (TMH) were evaluated at baseline, and at 30, 60, 90 and 135 minutes post-application. Subjective reports of comfort were also compared.

Results: Treated and control eyes were not significantly different at baseline ($p>0.05$). Post-application, LLG increased significantly, at 30 and 60 minutes, only in the treated eyes ($p=0.005$). NIBUT also increased significantly in the treated eyes only ($p<0.001$), at 30, 60 and 90 minutes. TMH did not alter significantly ($p>0.05$). Comfort improved relative to baseline in 46% of treated and 18% of control eyes, respectively, at 30 minutes post-application. Of those expressing a preference in comfort between the eyes, 68% preferred the liposomal spray.

Conclusions: Consistent with subjective reports of improved comfort, statistically and clinically significant improvements in lipid layer thickness and tear film stability are observed in normal eyes for at least an hour after a single application of a phospholipid liposomal spray.

Introduction:

The term ‘dry eye’ describes an ocular surface disorder of varying aetiology, but with common features including tear film hyperosmolarity and instability, visual disturbance, and symptoms of grittiness, burning and irritation.[1] It is a common disorder, with a reported prevalence of between 5% and 35%, depending on the age of the population studied and the diagnostic criteria used.[2] (DEWS epidemiology subcommittee) Dry eye is typically described as either aqueous-deficient or evaporative in origin, but these aetiologies are not mutually exclusive, and increased evaporation has been reported to be the more significant factor, contributing to dry eye signs and symptoms in as many as 78% of patients.[3-5]

The lipid layer of the tears has long been recognised to play an important role in inhibiting tear film evaporation.[6] Interfometrically, individuals without a visible lipid layer, or with a non-confluent lipid layer, exhibit a four-fold higher rate of tear film evaporation than those with a continuous lipid layer, irrespective of the lipid layer thickness.[7] Arising primarily from the meibomian glands of the eyelid, the lipid layer is a complex structure with a thin, inner polar layer, interfacing with the aqueous phase and reducing surface tension, and a thicker, outer, non-polar layer which is believed to inhibit tear evaporation.[8, 9] Meibomian gland dysfunction results in abnormal lipid production and has been identified as one of the major causes of ocular discomfort and ocular surface abnormality.[10-12]

Improved treatments for dry eye continue to evolve but, at the current time, artificial tear supplementation remains the primary therapy. Traditionally, the focus of such therapies has been to augment tear film volume to compensate for aqueous insufficiency but more recently, attention has been directed towards creating supplements that address deficiency in the tear film lipids. In the last decade, researchers have reported significant reductions in tear evaporation and improvements in lipid layer thickness with topical lipid emulsion eye drops containing neutral oils and castor oil.[5, 13-15]

In recent years, a phospholipid liposomal spray developed in Germany (Tears Again®, Optima Pharmazeutische GmbH) has been generating interest as a potential therapy for evaporative dry eye. The spray is applied to the closed eyelids, and the liposomes migrate, via the lid margins, into the tear film. Improvements in symptomatology, visual acuity, eyelid margin inflammation, tear production and lid parallel conjunctival folds have been documented with use of the lipid spray in patients with dry eye,[16-18] in contact lens wear[19] and following cataract surgery.[20] The observed changes are attributed to improvements in the lipid layer, however, to the best of our knowledge, there is no documented evidence of changes in the appearance of the tear film lipid layer, following application of the liposomal spray, to support these claims. This study was designed to investigate the effect of a single application of the liposome spray on the pre-ocular tear film in a group of normal and mildly symptomatic dry eye subjects.

Methods

The subject group comprised 22 non contact lens wearing individuals (12 males, 10 females) with a mean age of 33.5 ± 7.1 years (age range 24 to 46 years). There was no history or evidence of systemic or ocular disease other than self-reported borderline dry eye in four subjects, according to McMonnies Dry Eye Questionnaire.[21]

Lipid patterns and tear film stability were assessed non-invasively with the Tearscope Plus™ (Keeler Ltd, Berkshire, UK). Lipid patterns were graded according to the Guillon classification,[22] based on the predominant pattern visible during a 60 second examination-period with unrestricted participant blinking. The patterns were recorded as absent, open meshwork, closed meshwork, flow, amorphous or (evenly distributed) coloured fringes, corresponding to increasing lipid layer thickness, and assigned a lipid layer grade (LLG) of 0 to 5, respectively, for the purpose of analysis.

Use of the fine grid insert with the Tearscope Plus™ facilitated measurement of the non-invasive break-up time (NIBUT). Subjects were instructed to blink and then to refrain from blinking while the examiner observed the reflected grid pattern. NIBUT was recorded as the time between the blink and the first sign of a distortion or disruption in the reflected grid pattern. If the subject blinked prior to signs of break-up, the measurement was repeated. A mean of three measurements was calculated. Tear meniscus height (TMH) was evaluated from calibrated, 10x magnified digital video images collected at baseline and at each subsequent time point with a CSO (CSOphthalmic, Florence, Italy) digital slit lamp.[23]

The study was prospective, controlled, double-masked and randomised in design. LLG, NIBUT and TMH were evaluated for both eyes of each subject at baseline. Following a single application of the liposomal spray (Tears Again, currently marketed in UK as Optrex ActiMist™) to one eye and an identical volume (0.11 ± 0.01ml) of saline spray to the contralateral eye, from a distance of 10cm and with the aid of a septum to minimise the risk of inter-ocular contamination, LLG, NIBUT, TMH and subjective comfort were recorded at 30 minutes, 60, 90 and 135 minutes. A computer-generated randomisation schedule determined the application order and the eye to which each spray was applied for each participant. An investigator remote to the clinical evaluations applied the sprays (investigator-masked) and subjects' eyes remained closed throughout the application process to ensure participant-masking.

Subjective comfort was established at each time point by asking subjects, firstly, to relate their comfort to baseline in each eye (more comfortable / less comfortable / no different) and, secondly, to express a preference for their more comfortable eye (right eye / left eye / no difference). Data collection occurred over a period of two days during which environmental conditions remained stable at 25.4±0.3°C and 57.0±1.7% relative humidity. All measurements were collected at least 2 hours after wakening to minimise closed eye tear film effects.[24]

Research was conducted in accordance with the Declaration of Helsinki, with local ethical approval from Aston University Ethics Committee. Each subject was required to provide informed written consent prior to participation.

Statistical analysis

Statistical analysis was performed with SPSS v.16.0. Normally distributed continuous data underwent parametric statistical analysis. Normality was confirmed for the TMH data (Kolmogarov-Smirnoff, $p>0.05$), and for the positively skewed tear film stability (NIBUT) data following logarithmic transformation. Ordinal lipid data were analysed with non-parametric tests. A p-value of <0.05 was considered statistically significant.

Results

Prior to application of the liposomal spray, there were no significant differences in lipid characteristics, NIBUT or TMH between the treated and control groups (Mann-Whitney U, p=0.508; Unpaired Student t, p=0.672 and 0.815, respectively). Mean or median pre- and post-application values for the measured parameters across the time points are summarised in Table 1.

Post-application, significant differences in lipid layer thickness were observed across the time-points in the treated eyes (Friedman, p=0.005) but not in the control eyes (Friedman, p=0.121). Post-hoc testing in the treated eyes confirmed significant increases in lipid thickness, relative to baseline, at the 30 and 60-minute time points, but not at 90 or 135 minutes. Median lipid layer thickness categories across the time points are shown for the treated and control eyes in Figure 1. Significant differences in lipid patterns between the treated and control eyes were established at the 30, 60 and 90-minute time points (Wilcoxan, p=0.0016, p=0.0018, p=0.348, respectively) but not at baseline or 135 minutes (p=0.1597 and p=0.9998, respectively).

Repeated measures ANOVA demonstrated that tear film stability (NIBUT) varied significantly across the measurement period in the treated eyes ($p<0.001$), but not in the control eyes (p=0.925). Scheffe post-hoc testing in the treated eyes indicated that NIBUT was significantly increased at the 30, 60 and 90 minute time-points ($p<0.05$ in all cases), but returned to baseline levels by 135 minutes post-application. Figure 2 shows the variation in tear film stability across the measurement period in both treated and control eyes. Significant differences in NIBUT between treated and control eyes were established at the 30, 60 and 90-minute time points (Paired Student t, p=0.0001, p=0.0003, p=0.018, respectively) but not at baseline or 135 minutes (p=0.1684 and p=0.9266, respectively).

TMH was not found to vary significantly across the time-points in either the treated or control eyes (repeated measures ANOVA, p=0.058 and p=0.080, respectively), nor between the treated and control eyes at any time-point ($p>0.05$).

Comfort relative to baseline: Subjectively, improved comfort relative to baseline was noted by over 70% of subjects in the treated eye, maximal at the 30-minute time-point. By 90 minutes post-application, 80% of subjects were unaware of a difference in comfort relative to baseline. Approximately 18% of subjects reported increased comfort in the control eye, relative to baseline, across all time-points (Figure 3).

Subject Preference: At 30 minutes post-application, over 50% of subjects reported superior comfort in the treated eye compared with the control eye (Figure 4). No difference between the treated and control eyes was reported by 60 minutes post-application. Approximately one quarter of subjects recorded a preference for saline at each time point.

Discussion

Meibum contains a multitude of lipid species, 92% of which are neutral lipids and 8%, polar lipids.[11] The polar lipids consist of 70% phospholipids, the most predominant of which, comprising 38% of the phospholipid component, is phosphatidylcholine.

Polar lipids and, in particular, phospholipids are recognised to be important components within the tear film for their role in surface monolayer formation, and for their surfactant properties. In order for the hydrophilic aqueous-mucin phase of the tear film to support a hydrophobic non-polar lipid layer on the surface, an interposing surfactant layer is necessary.[9, 11] The polar lipids are understood to fulfil this role, not only facilitating apposition of these naturally repellent layers, but also functioning as a matrix upon which the superficial non-polar layer can form. Deficiency of these components prevents formation of a stable, continuous lipid layer, which, in turn, causes increased tear evaporation rates.[7, 11]

Increasing the levels of the natural meibomian lipids by warm compress treatment,[25] digital expression[26] or latent heat[27] can increase lipid layer thickness and tear film stability. However, these techniques are time-consuming and may require considerable effort. Artificial supplementation has the potential to offer a convenience that cannot be afforded by traditional methods.

The commercially available liposomal spray (Tears Again®) represents a novel delivery system, in which the major phospholipid, phosphatidylcholine, is delivered in a stable form (liposomes) to the closed eyelid and from there, migrates across the eyelid margins to combine with the natural tear film. Observation of the tear film following application of the liposomal spray combined with fluorescein, for visualisation purposes, has clearly demonstrated migration of the solution from the closed eyelids into the tear film after only a few blinks.[16] Liposomes are microscopic spherical vesicles, which form when hydrated phospholipids become organised, with consistent head-tail orientation, into circular sheets.[28] The sheets combine to form a phospholipid bi-layer membrane, which encapsulates aqueous-soluble material within aqueous to create a phospholipid sphere. As the liposomes are held together by hydrophobic interactions, they remain stable in aqueous solvents.[18]

Improvements in a number of tear film and ocular surface parameters, most significantly in the level of eyelid margin inflammation, have been reported with use of the liposomal spray.[16-18] This is presumed to be as a result of improvement of the tear lipid layer but, to date, the effect of application of the spray on the appearance of the lipid layer has not been described.

In the current study, it was observed that application of the liposomal spray caused a significant increase in the thickness of the tear film lipid layer for up to 60 minutes post-application. An improved polar lipid layer is believed to facilitate support of a thicker non-polar layer.[9, 11] Phospholipids are fundamental constituents of the polar lipid layer, understood to afford vital surfactant properties.[29]

Inclusion of a control (saline) in the study, enabled confirmation that lipid increases were a result of the liposomal spray application and not from increased meibomian gland secretions resulting from forceful blinking. Participant and examiner masking enabled comparison of both objective and subjective data following spray administration.

A significant increase in tear film stability, evaluated non-invasively, was also measured up to 90 minutes post-application in this double-masked study. An association between lipid layer grade and tear film stability has been identified previously, with thicker lipid layers associated with increased tear film stability and a reduced inter-blink period.[7] The increased tear film stability following application of the liposomal spray in the current study is consistent with the findings of previous longer-term studies, where tear break-up time has been shown to increase significantly.[16, 18]

One of the main disadvantages of artificial tear supplements is frequently quoted to be the short retention time, with many supplements providing symptomatic relief for only several minutes. Evidence of the beneficial effects of increased lipid thickness and tear film stability for periods of over an hour shows promise for this liposomal product.

Somewhat surprisingly, given the asymptomatic nature of the vast majority of participants prior to involvement in the study, comfort was reported to be improved relative to baseline, in over 45% of eyes treated with the liposomal spray, but less than 20% of the eyes subjected to the saline spray. This improved comfort has been reported in other studies of the liposomal spray, and might be further increased in a study of individuals symptomatic of dry eye at the outset. Indeed, improved comfort has been reported in a number of other studies of the liposomal spray,[16, 18-20] although not all were participant masked and therefore may have been subject to bias. Favourable feedback on the liposomal spray in the current study is supported by anecdotal clinical evidence suggesting that patients appreciate the convenience of the modality, the ease of administration compared with eye drops, and often tolerate the spray better than alternative supplements, especially gels, due to the absence of an adverse effect on vision with the spray.[30]

A limitation of the current study was the absence of significant dry eye signs and symptoms in the subject group. A similar study in patients with evaporative dry eye associated with reduced lipid availability is currently underway to determine the potential for improvement of tear film quality and symptomatic relief in this subject group.

Although the potential benefits of artificial lipid supplementation were described as early as 1990,[31] measurable benefits have become evident only relatively recently, with the formulation of a number of lipomimetic products, capable of delivering lipid components in a stable and functional form. While improving the quality and increasing availability of the natural tear film lipids through traditional and innovative eyelid therapies should not be underestimated, the availability of artificial lipomimetic products with proven benefit, in drop or spray form, provides a welcome addition to the armament of the clinician involved in the management of dry eye.

Conclusions

Significant improvements in tear film stability and lipid layer thickness can be achieved in normal eyes for between 60 and 90 minutes following a single application of a phospholipid liposomal spray to the closed eye.

Figure legends

Figure 1: Distribution of lipid patterns at each time point for the (a) treated and (b) control eyes. Lipid patterns are graded in order of thickness from absent [Abs], through open meshwork [M(o)], closed meshwork [M(c)], flow [F], and amorphous [Am] to evenly distributed coloured fringes [CF]. Asterisks (*) denote significant differences from baseline.

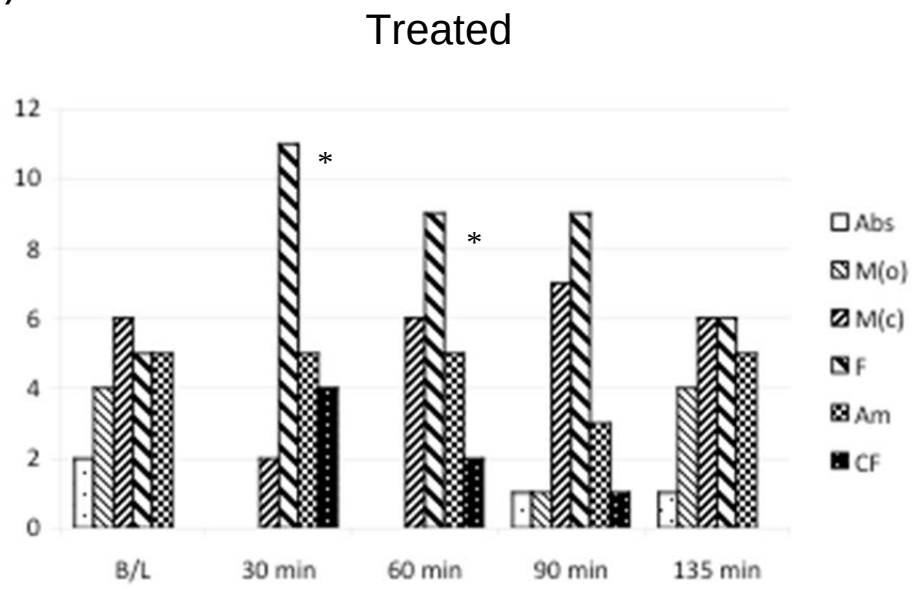
Figure 2: Mean non-invasive break-up time (NIBUT) across all time points for the treated and control eyes. Error bars show NIBUT standard deviation and asterisks (*) highlight significant differences in NIBUT from baseline.

Figure 3: Reported comfort relative to baseline, in the treated and control eyes, at each post-application time point.

Figure 4: Subjective preference between the treated and control eyes, post-application, with regard to comfort across at each time point.

Figure 1

(a)



(b)

Control

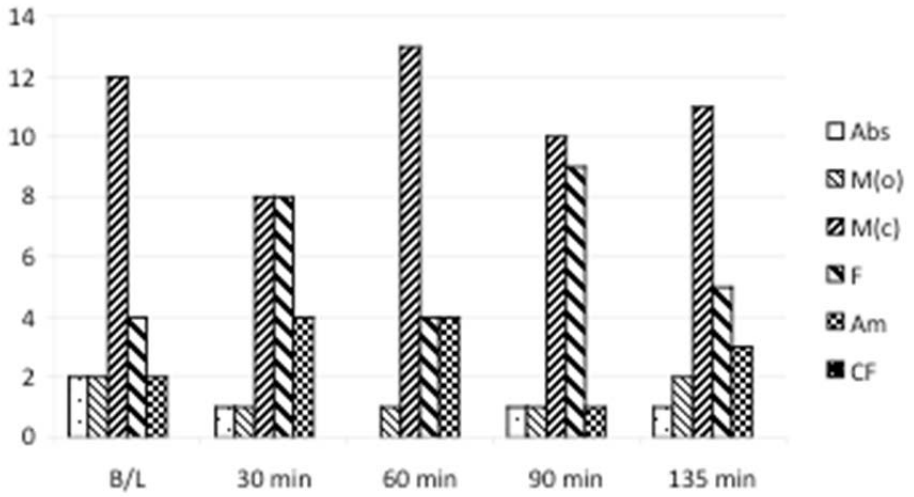

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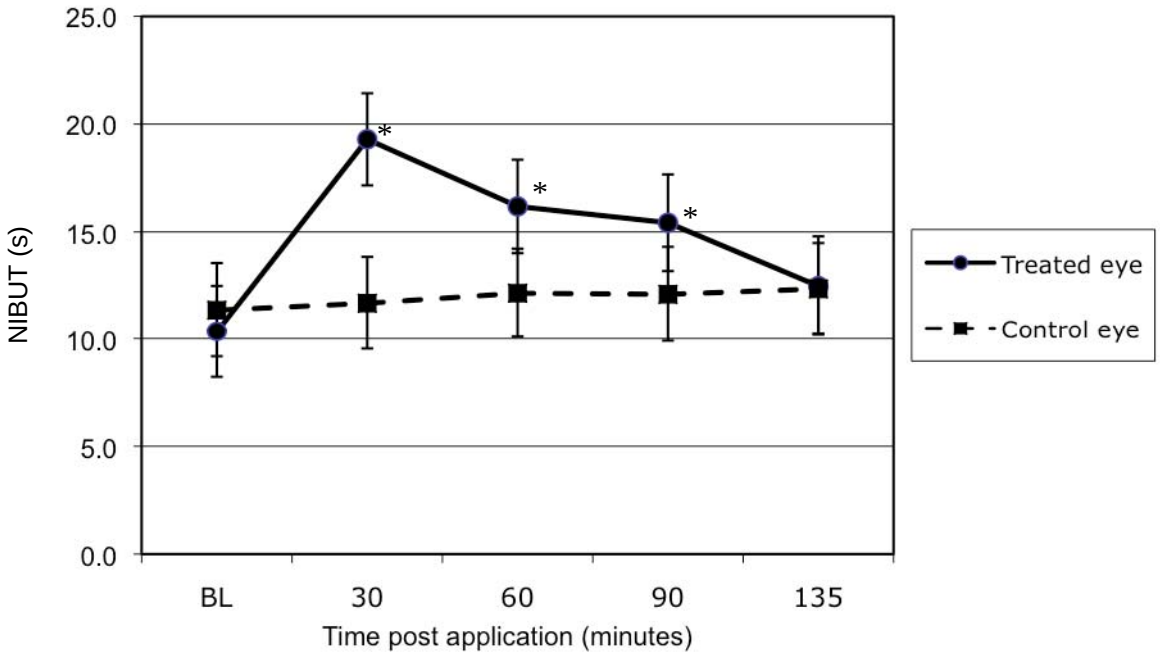
Figure 2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Time post application (minutes)
Treated eye
Control eye

Figure 3

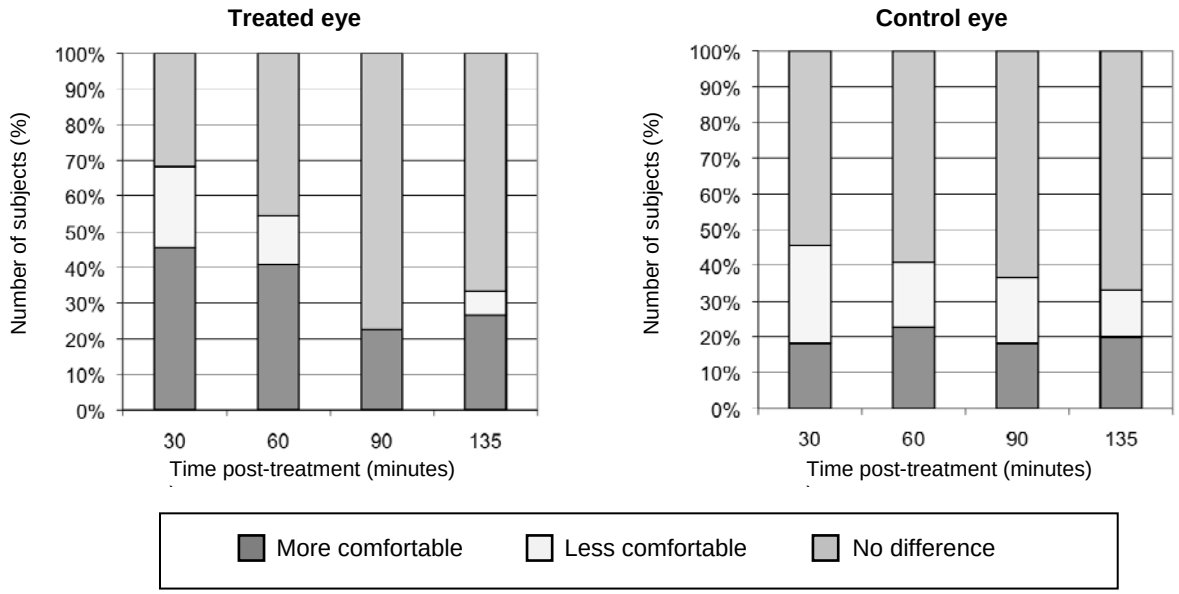

Figure 4

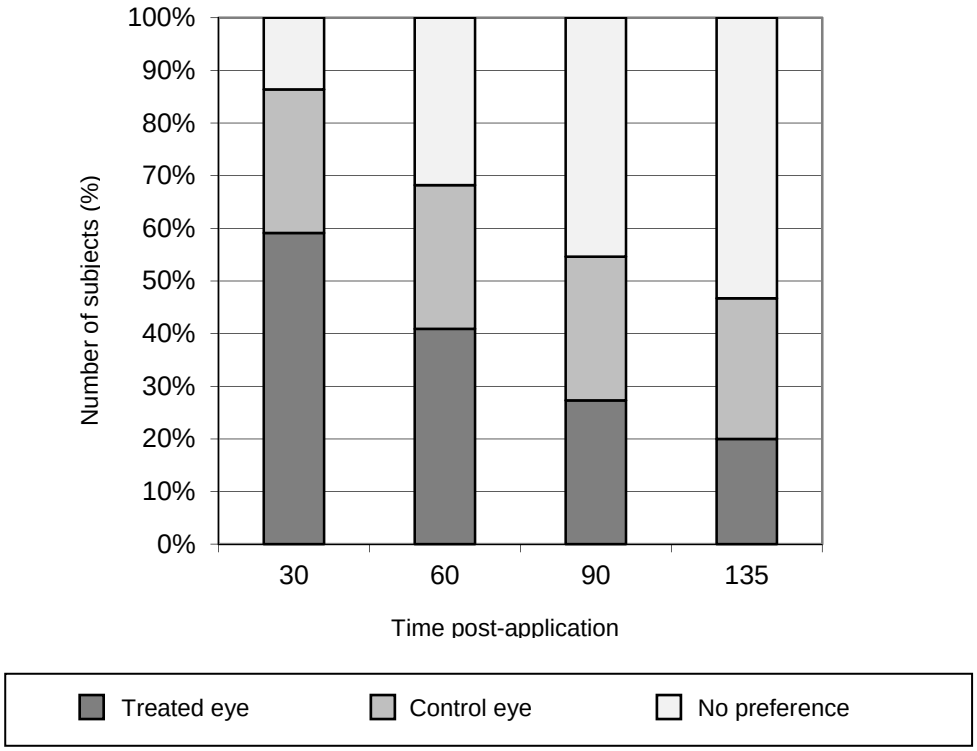

Table Legend

Table 1: Mean (or median) pre- and post-application values for the measured parameters across all time points

		Pre-application	Time post-application (minutes)			
	Eye	**Baseline**	**30**	**60**	**90**	**135**
Lipid grade median (min, max)	T	2 (0, 4)	3 (2, 5)	3 (2, 5)	3 (0, 5)	2 (1, 4)
	C	2 (0, 4)	3 (0, 4)	2 (1, 4)	2 (0, 4)	2 (0, 4)
NIBUT (s) (mean ± sd)	T	13.1 ± 8.8	24.3 ± 14.8	22.0 ± 12.2	23.0 ± 13.7	17.6 ± 17.1
	C	15.5 ± 13.2	14.2 ± 9.0	16.6 ± 12.0	17.0 ± 11.7	15.5 ± 9.4
TMH (mm) (mean ± sd)	T	0.39 ± 0.13	0.38 ± 0.10	0.38 ± 0.11	0.35 ± 0.10	0.36 ± 0.10
	C	0.40 ± 0.12	0.37 ± 0.10	0.38 ± 0.12	0.36 ± 0.13	0.37 ± 0.12
Comfort relative to baseline (% better)	T	-	45.5	40.9	22.7	26.6
	C	-	18.2	22.7	18.2	20
Comfort relative to baseline (% worse)	T	-	22.7	13.6	0	6.7
	C	-	27.3	18.2	18.2	13.3
Comfort T vs C eye (% prefer T)		-	59.1	40.9	27.3	20
Comfort T vs C eye (% prefer C)		-	27.3	27.3	27.3	26.7
Comfort T vs C eye (% equal)		-	13.6	31.8	45.4	53.3

Key: min = minimum, max = maximum, NIBUT = non-invasive break up time, T = treated eye, C = control eye, sd = standard deviation

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