Effect of One Overnight Wear of Orthokeratology Lenses on Tear Composition

CAMUS KAR MAN CHOY, BSc(Hons), PhD, PAULINE CHO, BOptom, PhD, IRIS FRANCES FORSTER BENZIE, DPhil, FIBMS, and VINCENT NG, BSc(Hons), PhD

Department of Optometry and Radiography (CKMC, PC, VN) and School of Nursing (FB), The Hong Kong Polytechnic University, Kowloon, Hong Kong SAR, China

ABSTRACT: Purpose. To evaluate the effect of one night of orthokeratology lens wear on ocular surface health based on changes in tear components, including ascorbate, urate, lactate dehydrogenase (LD), lactoferrin, lipocalin, lysozyme, secretory immunoglobulin A (sIgA), and serum albumin. Methods. Changes in tear components in eight healthy young men before and after 7-h overnight ortho-k lens wear were studied. Subjects attended on two separate occasions during a 1-week period, on one occasion wearing lens overnight and on the other wearing no lens. Tears (yawn-induced) were collected by capillary tube before lens fitting and on awakening. Tear ascorbate and urate were measured by high-performance liquid chromatography; LD was measured by a commercial kit method; tear proteins were measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Results. Ascorbate, sIgA, albumin, and LD increased significantly overnight with and without overnight lens wear (p < 0.05); however, no significant increases were found in tear urate, lactoferrin, lipocalin, or lysozyme (p > 0.05). Without lens wear, tear ascorbate, sIgA, albumin, and LD increased by 21%, 34%, 9-fold, and 13-fold, respectively (p < 0.05). With ortho-k lens wear, significant flattening of the apical curvature was observed as expected, and the increases in tear ascorbate, sIgA, albumin, and LD (increases were 56%, 76%, 13-fold, and 14-fold, respectively) were significantly (p < 0.05) greater than with no lens. There was significant correlation seen between changes in albumin and LD with (r = 0.762; p = 0.037) and without (r = 0.738; p = 0.046) ortho-k lens wear. Conclusions. The result of tear ascorbate suggests that corneal cell disturbance is small after one night of ortho-k lens wear. The marked increases in albumin and LD suggest that the ocular surface is under additional hypoxic stress during overnight ortho-k lens wear. (Optom Vis Sci 2004;81: 414-420)

Key Words: tears, cornea, ascorbate, protein, lactate dehydrogenase, orthokeratology

Myopia affects 25% of the white population and an even greater proportion of the Asian population. Spectacles and contact lenses are the most common methods of myopia management. Laser-assisted in situ keratomileusis (LASIK) and orthokeratology (ortho-k) are alternative avenues of myopia treatment. LASIK is a surgical procedure intended to permanently change the shape of the cornea using an excimer laser, whereas ortho-k involves a contact lens-induced temporary change (flattening) in corneal curvature that produces short-term reduction in myopia. The introduction of reverse geometry lens and rigid gas-permeable (RGP) lens materials has led to a more proactive, overnight ortho-k approach being used to obtain accelerated results. However, this approach has provoked some controversy, particularly in terms of ocular surface health during overnight wear. The effects of overnight contact lens wear on corneal health (in terms of edema and fluorescein staining) can be assessed by slitlamp examination, however, this is not a sensitive technique. A more sensitive laboratory approach used previously involves measurement of changes in tear protein concentration and changes in the cytosolic enzyme lactate dehydrogenase (LD). Because intracellular LD activity is high, corneal cell leakage or cell death results in massive release of this large tetrameric protein structure into the tear fluid. Several investigators have measured tear LD activity as an index of the status of the corneal epithelial surface (Table 1). The variation between methods and units used makes it difficult to directly compare the results from different studies, however, there are consistent findings that tear LD increases with anterior ocular surface irritation. For example, Schirmer strip contact with the lower lid increased tear LD activity.

Optometry and Vision Science. Vol. 81, No. 6, June 2004
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<tr>
<th>Author</th>
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<td>Kahan and Ottovay</td>
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<td>van Heeringen and Glasius</td>
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<td>MacKay et al.</td>
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<td>Fullard and Carney</td>
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<td>Tear LD activity varied with time, being highest in early morning. Diurnal variation or effect of hypoxia suggested.</td>
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<td>Collected at 10am</td>
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<td></td>
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<td>3.73 4.51 log units</td>
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<td></td>
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<td>Capillary tube</td>
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<td>High O$_2$ soft lens</td>
<td>3.92 4.80 log units</td>
<td>Tear LD activity was affected by different lens oxygen permeability and type.</td>
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<td>4.24 4.87 log units</td>
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<td>19 337 units/liter</td>
<td>Tear LD activity was affected by both diurnal variation and ortho-k lens wear. The effect of hypoxia (due to eye closure or contact lens wear) suggested</td>
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<tr>
<td></td>
<td>8</td>
<td>Overnight orthokeratology lens wear</td>
<td>33 618 units/liter</td>
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up to 10-fold, and LD increased also with contact lens wear. No similar study has been performed to determine the change of LD activity in human tears as a result of wearing ortho-k lenses, but this approach could help assess potentially deleterious corneal effects of ortho-k lens wear.

In addition to LD, corneal cells are also rich in ascrobate, and we hypothesize that increased tear ascrobate may be an even more sensitive biomarker of subclinical corneal or conjunctivul leakage. Ascrobate is small in size compared with LD and is likely to leak into the tears at an earlier stage of cell damage. However, we are not aware of any reports on changes in tear ascrobate levels with contact lens wear. This was a point of interest in this study. Previous studies have also measured the concentrations of other tear proteins to investigate baseline levels and to determine the effect of closed eye condition and contact lens wear. Results indicated that the tear concentrations of secretory immunoglobulin A (sIgA) and albumin increased dramatically in the closed eye condition, and the increase was compounded by overnight contact lens wear; however, no significant changes in lactoferrin, lipocalin, or lysozyme concentrations were found. Lactoferrin, lipocalin, and lysozyme are secreted by the lacrimal glands rather than entering the tears because of leakage from cells. Increases of sIgA and albumin concentrations are likely to reflect a constitutive change in tear fluid during eye closure compared with a neurologically regulated lacrimal secretion in open eye condition. The function of sIgA in tear fluid is to protect the anterior eye by acting as an immunologic barrier and in albumin is the result of leakage from dilated vessels. Therefore, the increase of sIgA and albumin concentrations in tears may indicate that a closed eye environment induces a subclinical inflammation and that this condition is exaggerated by overnight contact lens wear.

Because of the increasing popularity of the use of ortho-k for myopia management and its application in children in some Asian countries, it is important and timely to determine the safety of this treatment in addition to its efficacy. We propose that monitoring tear composition offers a useful biomarker approach to assess ocular changes induced by overnight ortho-k lens wear. This was the focus of this study, in which the effect of a single night of ortho-k lens wear on tear components was investigated. Specific components measured in tears pre- and post-ortho-k lens wear included ascrobate, LD, lactoferrin, lipocalin, lysozyme, sIgA, and albumin.

**METHODS**

Experiments were performed to determine the changes in ascrobate, LD, lactoferrin, lipocalin, lysozyme, sIgA, and albumin in tear fluid from healthy young adults before and after a single overnight (7 h) use of ortho-k lens. The Ethics Subcommittee of The Hong Kong Polytechnic University approved this study, and all procedures involving human subjects complied with the Declaration of Helsinki as revised in 2000.

Ten subjects (Chinese men) aged 22 to 30 years were recruited with their informed consent. All subjects were asymptomatic. None were smokers or users of vitamin supplements, and none were contact lens wearers. Subjects in this crossover study attended our clinic on two separate occasions during a 1-week period. On both occasions they were provided with the same food and bever-

age 4 h before the first tear collection. Our previous studies have shown return to baseline of tear ascorbate by 4 h after ingestion of vitamin C. Five subjects were ortho-k lenses on the first occasion, and five subjects were ortho-k lenses on the second visit. Each subject acted as own control, wearing no lens on one overnight trial. Tear samples (induced by yawn) were collected just before insertion of the lenses (Fargo trial lenses: HDS100, Dk/100 × 10^-11 cm²/s ml O₂/m l × mm Hg; central thickness, 0.2 mm (G.P. Specialists, Ltd., Phoenix, AZ)). Volunteers underwent a routine eye examination for refraction and ocular health at the Hong Kong Polytechnic University Optometry Clinic. A qualified optometrist performed the examination and fitting of lenses following our routine protocol. All lenses fitted showed adequate movement on blink and good centration—about 4 mm minimal central bearing and light midperipheral bearing. No clinically significant lens binding was observed in any of the subjects on awakening. All trial lenses used were designed to reduce myopia by 2 D. After insertion of the trial lenses (both eyes), the subjects were requested to keep their eyes closed from then until going to sleep, although eyes were not taped shut. Another tear sample was collected as soon as possible after the subjects awakened in the morning and before lens removal. Slitlamp examination after fluorescein staining was also used to assess corneal integrity before and after the overnight trial periods. We also used Medmont E300 cornet topographer (version 3.90, Medmont Pty. Ltd., Camden, Australia) to measure the apical curvature of the cornea of each subject before and after overnight ortho-k lens wear.

Because of resource limitations, the researcher who collected and analyzed the tears was not masked with respect to whether the subject had worn lenses overnight. However, it is unlikely that bias could have been introduced into the biochemical analyses because all samples collected on each study day were processed in parallel with automated methods of analysis used.

For tear analysis, about 40 µl yawn-induced reflex tears were collected from both eyes using 20 µl disposable capillary tubes (Drummond Scientific Co., Broomall, PA). To minimize the effect of different tear flow rate for each subject on different occasions, the time for tear collection was recorded, and the data of eight subjects who had similar tear flow rates in pre- and post-lens wear samples were selected for analysis. The within-subject variation in tear flow rate was not in any particular direction.

Ascrobate concentration and LD activity in the tear samples were measured within 1 h of tear collection. Ascrobate was measured using a Waters Millennium high-performance liquid chromatography (HPLC) system (Waters Alliance, Milford, MA) as described previously. LD activity was measured using a commercial kit (LDH/SDH Unimate 3, Roche Diagnostic Ltd., Basel, Switzerland) on a Cobas Fara centrifugal analyzer (Roche). Tear urea was also measured (by HPLC) to investigate possible evaporation effects: Urate is not concentrated within cells, and we have found that urate concentration does not increase with ocular surface irritation.

Qualitative and quantitative analyses of the major tear proteins were performed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Followed by scanning densitometry as described previously. In brief, SDS-PAGE was performed on Bio-Rad MiniProtein II Cell (Bio-Rad, Hercules, CA) according to the discontinuous buffer system of Laemmli. After SDS-
PAGE, the gels were stained with Coomassie blue (R-250) and scanned by a Bio-Rad image densitometer (GS-670). The concentrations of the major tear proteins were determined with reference to the standard curves plotted from purified human proteins—slgA, lactoferrin, albumin, lipocalin, and lysozyme. All tear samples obtained from a single subject were analyzed in the same gel to avoid any between-gel variability.

For statistical analysis, the sample size was determined by power analysis, and sample size was suggested to be 10. For sample size equal to eight, the probability of type I error (α) is 0.05, and the probability of type II error (Β) is 80% when the true difference equals the expected mean difference of ≥8 μM (for ascorbate), ≥1 mg/ml (for IgA), ≥0.1 mg/g (for serum albumin), or ≥30 U/l (for lactate dehydrogenase). Nonparametric tests were used to analyze the data in view of the small sample size. The Friedman test with a post hoc test (Dunn's multiple comparisons test) was performed to compare levels of tear variables before and after each experiment. Wilcoxon matched-pairs signed rank test was used to compare the before and after differences in variables between subjects with and without ortho-k lens wear. The Mann-Whitney test was used to investigate the before and after differences in variables between subjects with and without positive fluorescein staining. Spearman rank correlation was used to investigate inter-relationships between the overnight changes in the variables of interest.

RESULTS

The median and range of the variables of interest in tears with and without ortho-k lens wear are shown in Table 2. The concentrations of ascorbate, slgA, albumin, and LD activity increased significantly (p < 0.05) overnight irrespective of whether ortho-k lens were worn; however, no significant increase was found in the tear urate, lactoferrin, lipocalin, and lysozyme concentrations (p > 0.05). Without ortho-k lens wear ("control"), the tear ascorbate and slgA concentrations increased overnight by an average of 21 and 34%, respectively (p < 0.05), whereas albumin concentration and LD activity increased markedly by an average of 9- and 13-fold, respectively (p < 0.05). With ortho-k lens wear ("test"), the same variables increased significantly (p < 0.05), and the increases were significantly larger than without ortho-k lens wear (Table 2). Tear ascorbate and slgA concentrations increased after overnight ortho-k lens wear by 56 and 76%, respectively, and tear albumin concentrations and LD activity increased by 13- and 14-fold, respectively. As expected, a significant flattening of the cornea was observed (p < 0.05; mean ± SD change in apical radius, 0.20 ± 0.08 mm; 16 eyes) with ortho-k lens wear; however, the overnight change in the apical curvature with ortho-k lens wear was not significantly correlated with the increase in tear ascorbate (r = 0.02; p = 0.98), slgA (r = 0.33; p = 0.43), albumin (r = 0.60; p = 0.13), or LD (r = 0.24; p = 0.58). There was a strong direct correlation between overnight changes in tear albumin concentration and those in LD activity (r = 0.76; p = 0.047) and without (r = 0.74; p = 0.046) ortho-k lens wear. No significant correlation was found among increases in the other variables (p > 0.05).

No subject exhibited fluorescein staining before the overnight trial periods; however, mild corneal fluorescein staining (≤grade 1,
Efron's scale was seen on awakening in five of eight subjects after overnight ortho-k lens wear and in three of eight subjects without ortho-k lens wear. The change of tear ascorbate concentration in subjects with fluorescein staining was significantly (p < 0.05) higher than those without staining. This was true whether lenses were worn or not. However, the changes of sIgA and albumin concentrations and LD activity were not significantly different between subjects with and without corneal staining (p > 0.05; results not shown).

DISCUSSION

Recent studies have reported that the rate of epithelial cell sloughing is reduced in contact lens wear, and it was also suggested that contact lens wear might help to protect the cornea from mechanical damage during blinking and eye movement. Nonetheless, there is potential for any foreign body in close proximity to the cornea to affect corneal integrity. Of relevance here are recent reports of central epithelial thinning associated with ortho-k lens wear. This current study aimed to provide an objective, preliminary assessment of ocular surface changes induced by a single night of ortho-k lens wear by measuring overnight changes in tear composition.

The proteins lactoferrin, lipocalin, and lysozyme are secreted into tears by the lacrimal gland. Their concentrations do not change with variation of tear flow rate and change only with aging and disorders of the lacrimal gland. Our results showed that the concentrations of lactoferrin, lipocalin, and lysozyme were not significantly changed overnight whether lens were worn or not, indicating no abnormality or change of the lacrimal glands of our subjects. Interestingly, however, tear ascorbate, sIgA, and albumin concentrations and LD activity increased overnight regardless of whether ortho-k lenses were worn or not. The increases in these variables were statistically significantly larger after ortho-k lens wear compared with overnight changes with no lens. However, only the LD and albumin increases were markedly different; changes in tear ascorbate and sIgA concentrations were only slightly greater with overnight ortho-k lens wear than without. What could cause an overnight increase in these tear components? We suggest three possibilities: cellular leakage in ocular surface, an intrinsic diurnal rhythm, and a relatively hypoxic environment in the closed eye or with contact lens wear.

The small overnight changes seen in tear ascorbate concentration are interesting and contrast with the much larger increases seen in LD activity. The level of tear LD activity has been used in the past to investigate changes in the ocular surface, with a general assumption that tears LD is largely of corneal origin. The large increases seen in tear LD activity in the current study would appear to indicate that corneal involvement was significant. However, the ascorbate data refute this. Changes in tear ascorbate concentration were modest with and without ortho-k lens wear. Tear ascorbate concentration is about 20 μM, whereas ascorbate in corneal epithelial cells is about 35 times this level. Therefore, even slight integrity change of the ascorbate-enriched corneal cells would be expected to markedly increase tear ascorbate concentration, making ascorbate a potentially highly sensitive indicator of corneal integrity. Interestingly, the change of tear ascorbate concentration in this study agreed well with fluorescein staining assessment with larger increases in tear ascorbate concentration found in subjects with fluorescein staining. Based on these data, as well as the observation that large increases in LD activity were seen also without lens wear and that LD activity was not significantly different in subjects with and without fluorescein staining, it is unlikely that the increases of LD activity were related to the deleterious change in the cornea.

Our results also indicate that the concentration of urate in tear fluid after an overnight sleep showed no significant increase. Urate is an endogenous purine breakdown product and does not show such a large intracellular and extracellular differential in concentration as LD and ascorbate. Therefore, cell leakage would not be expected to cause a marked increase in the tear urate level; however, tear evaporation or intracellular osmotic changes affecting water flow would cause a change in tear urate concentration. Increased anaerobic metabolism in the cornea in a hypoxic (closed eye or contact lens wear) environment leads to increased intracellular lactate and consequent water influx from tears to the cornea, which in turn will cause, in addition to corneal edema, a slight increase in the concentration of tear constituents. Our data, which showed modest increases in urate only, indicate that the large LD changes seen cannot be accounted for by evaporation or loss of water from tears as a result of simple osmotic changes within corneal cells. However, these factors are likely to account for the slight increases seen in urate and ascorbate concentrations seen when no lens were worn. The overnight changes in urate were about 26% without lens wear, and this was similar to the change in ascorbate (about 21%) seen in subjects wearing no lens. However, the overnight change in urate with ortho-k lens wear was 56% (the changes in urate were about 40%). This could indicate some cellular leakage of ascorbate from corneal or conjunctival epithelial cells with overnight ortho-k lens wear. This is not unexpected because the presence of a foreign body in the eye is likely to cause some corneal leakage. However, the increase in tear ascorbate with ortho-k lens wear was small in absolute terms (10 μM) and indicates that corneal involvement, if any, was likely to be minor. This is reassuring given the increasing popularity of this myopia treatment in Hong Kong, especially in children.

A diurnal rhythm in tear LD was suggested by Fullard and Carney, who reported finding highest LD levels in tears on awakening. However, their data do not confirm a true diurnal rhythm in tear LD and are compatible with a hypoxia effect, as discussed by the authors. It is of relevance that tear and blood vessel permeability has been reported to increase in a relatively hypoxic environment in the closed eye, with an increase in efflux in LD. In addition, many published reports have shown that LD activity (as opposed to LD mass) also increases with contact lens-associated hypoxia. As a result of this leakage, and together with the reduction of the rate of tear production in sleep, LD will become more concentrated in tear fluid. In this current study, we found that tear albumin concentrations also increased overnight, and there was a strong and significant relationship between the increase in albumin concentration and the increase in LD activity. This correlation has been reported previously and suggests that the source of these two components is similar in the relatively hypoxic environment in the closed eye and with contact lens wear.

The concentration of sIgA in tears after an overnight sleep was significantly increased, with significantly larger increases seen in
orthok lens wear. The findings are in agreement with previous reports that showed that slgA concentration increases after sleep with or without contact lens wear. 19-21 It has been suggested that this may be a sign of subclinical inflammation in the closed eye or that the increase in slgA concentration confers greater tear film protection against pathogens. 19-21 This remains to be confirmed.

This study is the first one of a crossover design that has investigated tear components immediately before lens wear/sleep and on awakening after overnight sleep with and without lens wear. This design allowed direct comparison of the changes in tear fluid that occur normally in the closed eye environment overnight with those that occur in the presence of an orthok lens. Our results clearly show that one overnight wear of orthok lens lead to significant changes of tear ascorbate, slgA, albumin, and LD concentrations. This change is most likely caused by the presence of some corneal leakage and hypoxia after overnight orthok lens wear, but clinically the changes were slight and too small to be identified by slitlamp examination. Whether this is true for all contact lens types remains to be established. For extended RGP lens wear, it has been reported that lens with oxygen permeability about Dk/s of 50 x 10^-9 cm³/ml sec/cm Hg (similar to the lenses used in this study) is sufficient to avoid most of the hypoxia-related complications associated with hydrogen extended wear. 40 It would have been useful to have a direct comparison between changes of tear composition after overnight wear of different kinds of regular lenses, including RGP lenses, to see whether the orthok lens design has more pronounced effects than regular lenses. Additional study is warranted in this area and in assessing regular and long-term use of overnight orthok lenses.

In conclusion, the aim of this study was to investigate the effect of a single night of orthok lens wear on tear components. Based on the tear ascorbate results, which were similar with and without lens wear, we can conclude that the deleterious change in corneal integrity is not an issue of concern after one night of orthok lens wear. Although marked overnight changes in tear albumin and LD were found in this study, we suggest that these are related to hypoxia in the closed-eye environment because similar changes were seen also without lens wear. The effect of the single overnight use of orthok lenses on other tear components was slight. Such changes may also be found in overnight RGP (of same Dk/s) lens wear. We suggest that more studies should be done to determine the safety of overnight use of contact lenses in general.

ACKNOWLEDGMENTS

This work was financially supported by a research grant (A356) from The Hong Kong Polytechnic University.

Received November 13, 2003; accepted February 11, 2004.

REFERENCES


Camus K. M. Choy
Department of Optometry and Radiography
The Hong Kong Polytechnic University
Kowloon
Hong Kong SAR, China
e-mail: orvch3@inet.polyu.edu.hk