Effect of ambient temperature on the human tear film

Abusharha, Ali A.; Pearce, E. Ian; Fagehi, Raied

Published in:
Eye and Contact Lens: Science and Clinical Practice

DOI:
[10.1097/ICL.0000000000000210](https://doi.org/10.1097/ICL.0000000000000210)

Publication date:
2016

Document Version
Author accepted manuscript

[Link to publication in ResearchOnline](#)

Citation for published version (Harvard):
Abusharha, AA, Pearce, EI & Fagehi, R 2016, 'Effect of ambient temperature on the human tear film', *Eye and Contact Lens: Science and Clinical Practice*, vol. 42, no. 5, pp. 308-312.
<https://doi.org/10.1097/ICL.0000000000000210>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please view our takedown policy at <https://edshare.gcu.ac.uk/id/eprint/5179> for details of how to contact us.

Effect of ambient temperature on the human tear film

Ali. A. Abusharha¹, PhD, E. Ian Pearce², PhD, Raied Fagehi,¹ PhD

1. Department of Optometry and Vision sciences King Saud University, Saudi Arabia

2. School of health and life sciences, Glasgow Caledonian University, United Kingdom.

Correspondence

Ali.A. Abusharha

King Saud University, Riyadh 12372

FAX: 01413313387

Tel: 00966114693543

Email: aabusharha@ksu.edu.sa

E.Ian Pearce

70 Cowcaddence Road

Glasgow G4 0BA

FAX: 01413313387

Tel: 01413318201

Email: E.I.Pearce@gcu.ac.uk

Raied Fagehi

King Saud University, Riyadh 12372

FAX: 01413313387

Tel: 00966114693543

Email: rfagehi@ksu.edu.sa

Acknowledgement

The authors extend their appreciation to the college of Applied Medical Sciences Research Centre and the Deanship of Scientific Research at King Saud University for its funding for this research.

Effect of ambient temperature on the human tear film

Abstract

Purpose: During everyday life the tear film is exposed to a wide range of ambient temperatures. This study aims to investigate the effect of ambient temperature on tear film physiology.

Method: A Controlled Environment Chamber (CEC) was used to create different ambient temperatures (5°C, 10°C, 15°C, 20°C, 25°C) at a constant relative humidity of 40%. Subjects attended for two separate visits and were exposed to 25°C, 20°C and 15°C at one visit and to 10°C and 5°C at the other visit. The subjects were exposed to each room temperature for 10 minutes before investigating tear film parameters. The order of the visits was random. Tear physiology parameters assessed were tear evaporation rate, non-invasive tear breakup time (NIBUT), lipid layer thickness (LLT), and ocular surface temperature (OST). Each parameter was assessed under each condition.

Result: A three-fold increase in tear evaporation rate was observed as ambient temperature increased to 25 °C ($p=0.00$). The mean evaporation rate increased from 0.056 $\mu\text{l}/\text{min}$ at 5°C, to 0.17 $\mu\text{l}/\text{min}$ at 25°C. The mean NIBUT increased from 7.31 sec at 5°C to 12.35 sec at 25°C ($p= 0.01$). A significant change in LLT was also observed ($p= 0.00$), LLT median ranged between 20 to 40nm at 5 and 10 °C and increased to 40 and 90nm at 15, 20 and 25°C . Mean reduction of 4°C OST was observed as ambient temperature decreased from 25 to 5°C.

Conclusion: Ambient temperature has a considerable effect on human tear film characteristics. Tear evaporation rate, tear lipid layer thickness, tear stability and ocular surface temperature were considerably affected by ambient temperature. Chronic exposure to

24 low ambient temperature would likely result in symptoms of dry eye and ultimately ocular
25 surface disorders.

26 **Keywords:** Tear film, dry eye, environmental factors, ambient temperature.

27

Introduction

Tear film function and structure can be altered by many different factors. Some of these factors are internal disorders such as insufficient tear production, meibomian gland dysfunction, hormonal changes and autoimmune disease.¹ In addition, as the tear film is directly exposed to the ambient environment, tear film thinning and instability can be caused by external factors such as ambient temperature, humidity, air flow and pollution.^{2,3} Recently, researchers have shown an increased interest in the effect of the ambient environment on human body tissues.^{4,5} Symptoms including ocular itching, stuffy nose, dry throat, breathlessness, dry skin and headaches have been frequently reported by individuals working in adverse environmental conditions.^{6,7}

Ambient temperature is an external environmental factor that could affect the tear film. In addition to outdoor ambient temperature, the thermal conditions of commercial buildings and indoor workplace are not-well controlled. Unfortunately, to date, little attention has been paid to the relationship between the change in ambient temperature and tear film behaviour. One previous study has suggested that a relationship exists between tear film and atmospheric temperature.⁸ A significant difference in tear stability and production was found in normal subjects living in places characterized by a warm climate compared with those living in cold places.⁸ Another in vitro study investigating the effect of temperature on tear evaporation found that there was a threefold increase in evaporation rate as ambient temperature increased from 25 to 34°C.⁹ The relationship between ocular surface temperature and room temperature has been well documented.¹⁰⁻¹³ There has been little discussion about the effect of ambient temperature on tear film parameters. Most researchers to date tended to focus on the relation between OST and ambient temperature ignoring its effect on tear film parameters and the inter-relationship between these parameters. Therefore, the aim of this study was to determine

the effect of ambient temperature on ocular surface temperature and its effect on the ocular surface and tear film parameters. The inter-relationship between tear parameters and OST will be monitored at across range of different temperatures.

Method

Ethical approval was obtained from the Glasgow Caledonian University Human Ethics Committee. Subjects were healthy normals with no evidence of dry eye. Inclusion criteria was Ocular Surface Disease Index (OSDI) ¹⁴ score of less than 12 and NITBUT of more than 10 seconds using HIRCAL grid.¹⁵ Twelve healthy normal subjects (3 female, 9 male, mean $\pm$ Sd 29.4 $\pm$ 2.4 years) with no current ocular diseases or surgery were enrolled in this study.

As the control of ambient temperature and humidity was required during the experiment, a controlled environment chamber (CEC) (Weiss-Gallenkamp Ltd, Loughborough, UK) was used.¹⁶ The room temperature was set at 5 and 10°C on one visit. On the other visit, the CEC was set at temperatures of 15, 20 and then 25°C.

The subjects were divided into two groups (Group A and Group B). The order of visits was randomised in a cross-over design where the subjects in group A were exposed to a temperature of 25, 20 and 15°C and then exposed to 10 and 5° C ambient temperatures. Group B subjects were exposed to ambient temperature of 10 and 5°C, then at their second visit they were exposed to 25, 20 and 15°C. The relative humidity inside the CEC was maintained at 40% during the assessment of the tear film during the two visits. In order to minimize the possible implications of two visits, tear parameters assessments were conducted late morning and afternoon (after 11am) in both visits to avoid the effect of diurnal variation in ocular surface temperature.

A previous study investigating tear film parameters under different environmental conditions found a 6 to 10 minutes adaptation time was needed for the subjects before conducting ocular surface investigations.¹⁷ In this study, for the purpose of room temperature adaption, the subjects were exposed to each environmental condition for 10 minutes before starting investigations tear parameter.

The parameters assessed in this study were lipid layer thickness (LLT), tear evaporation rate (EVAP) non-invasive tear break-up time (NITBUT) and ocular surface temperature (OST). Tear break-up time and tear lipid layer thickness were assessed non-invasively by Keeler Tearscope Plus (Keeler Ltd, Windsor, UK). The Guillon and Guillon grading system was utilized to estimate the thickness of the tear lipid layer.¹⁸ Tear evaporation rate was measured using a Servo-Med Evaporimeter (Servo Med, Varberg, Sweden).¹⁹ A change in ocular surface temperature during exposure to the different ambient temperature was monitored with FLIR System ThermoCAM P620 (FLIR Systems, Surry, UK).¹⁶ A circle of approximately 4 mm diameter was placed at the estimated centre of the cornea and the mean temperature of this area was calculated. Thermal images of ocular surface were continuously recorded for one minute at frame rate of 30Hz. All temperature measurements were then exported to an Excel spreadsheet and 600 thermal values were selected with exclusion of the reading recorded immediately post-blink.

Variables were tested for normality using a Kolmogorov-Smirnov test. A repeated measure ANOVA and Tukey's post-hoc test were applied for normally distributed data while the ordinal and data with non-normal distribution were analysed using Friedman's test and post-hoc Wilcoxon rank-sum test. Correlation between tear parameters was assessed using Pearson's test and Spearman's test for the data with normal and non-normal distribution respectively.

97 **Result**

98 **Lipid layer thickness**

99 Changes in lipid layer thickness were found as temperature was altered (Figure 1A). The
100 median grade of lipid layer thickness observed was grade 2 (20 -40 nm) at 5 and 10°C and
101 increased to grade 3 (40- 90 nm) at 15, 20 and 25°C. Wilcoxon rank-sum test has shown that
102 the lipid layer thickness was significantly thinner at 5°C in contrast to other ambient
103 temperatures ($p<0.05$). Also a significant difference was observed at 10°C compared to lipid
104 thickness at 20 ($p=0.006$) and 25°C ($p=0.007$). No significant change in lipid layer thickness
105 was noticed when room temperature was changed from 20 to 25°C.

106 **Tear evaporation rate**

107 The box plot of tear evaporation indicates that, as the ambient temperature was increased, the
108 tear evaporation rate also increased (Figure 1B). A three-fold increase in tear evaporation was
109 observed as the ambient temperature increased from 5 to 25°C. The mean evaporation rate
110 was 0.056 $\mu\text{l}/\text{min}$ (20.11 $\text{g}/\text{m}^2/\text{h}$) at 5°C, but increased dramatically to 0.17 $\mu\text{l}/\text{min}$ (62.62
111 $\text{g}/\text{m}^2/\text{h}$) when the room temperature was raised to 25°C. Tukey's post-hoc test has been done
112 for evaporation rate data. Statistically significant differences were observed in evaporation
113 rate at 5°C when compared to 20°C ($p=0.013$) and 25°C ($p=0.001$).

114 **NITBUT**

115 Changes in tear film stability are shown in Figure 1C. At a room temperature of 5°C a
116 significant reduction was found in NITBUT compared to those values obtained at all other
117 temperatures (10, 15, 20 and 25 °C), ($p<0.05$). The mean NITBUT values were 7.31 sec and
118 12.35 sec at 5°C and 25°C respectively. Measurements of NITBUT were statistically

analysed using Tukey's post-hoc test. A significant change in NITBUT was observed at 10°C when compared to 20 ($p=0.002$) and 25°C ($p=0.001$).

Ocular surface temperature

Ocular surface temperature also showed a significant change ($p<0.05$). From the data in figure 1D it is apparent that there is a clear trend of decreasing OST as ambient temperature decreased. A reduction of 4°C OST was observed as ambient temperature decreased by 20° C (from 25 to 5 °C).

Correlation tests were applied to determine the relationship between tear parameters as temperature was altered. Data from all temperatures were combined and analyzed using either Persons's (R) for normally distributed data or Spearman's (ρ) correlation tests for ordinal and data with non-normal distribution. In Figure 2A a scatter plot shows a positive relationship between ocular surface temperature and evaporation rate ($r = 0.45$, $p<0.05$). Also, a significant correlation noted between evaporation rate and tear break-up time ($r = 0.32$, $p<0.001$) (Figure 2B). Tear evaporation was also showed a negative relationship with lipid layer thickness ($\rho = 0.43$, $p<0.001$) (Figure 2C). Also a significant correlation between lipid layer thickness and tear film stability was found ($\rho= 0.49$, $p <0.001$) (Figure 2D).

Discussion

The purpose of this study was to examine the effect of ambient temperature on tear film and the ocular surface. Tear stability, lipid layer thickness, evaporation rate and ocular surface were measured over a range of temperatures to evaluate the relationship between these parameters and temperature. In addition, the inter-relationship between these parameters was

examined to see how these are linked. This is an attempt to understand the relationship between these parameters in normal physiological conditions.

The evidence from this study suggests that the tear film lipid layer is affected significantly at low temperatures. It shows interference patterns characteristic of a thinner tear film after exposure to a temperature of less than 10°C. Hydrocarbon chains make up most of the lipid mass. It has been shown that lipid hydrocarbon structural order is affected by temperature.²⁰ Moreover, a relationship between lipid hydrocarbon chain order and meibomian lipid delivery to the lid margin has been found.²¹ The melting range of meibomian lipids has been shown to be between 32.50 and 35°C.^{22,23} Previous work has shown that meibomian lipids become thicker as the ocular surface and eyelid temperature drops below 33°C, which may impede the normal delivery of meibomian lipids to the ocular surface.²⁴ In addition to delivery, it has been shown that spreading over the tear film of meibomian lipids is also affected by temperature.²³

In the current study ocular surface temperature of less than 32 °C was observed after exposure to a room temperature of less than 10°C. Therefore, the changes in thickness and appearance of the tear film lipid layer observed in this study are likely to be due to variation in delivery and spread of meibomian lipid over the ocular surface.

A change in tear evaporation rate was observed when the ambient temperature changed. The evaporation rate increased as the temperature increased. The mean evaporation rate at 25°C was double that observed at 10°C, and triple the values recorded at 5°C. Box plots of evaporation rate at 25°C show that a quarter of participants had an evaporation rate characteristic of dry eye patients (evap>0.23µl/min²⁵). The lipid layer plays a critical role in controlling tear film evaporation. However, although a thin lipid layer was observed at 5 and 10°C, the evaporation rate was low in the cold environment, meaning that there may be

another factor apart from the meibomian lipid that could affect the evaporation of tears. Decreased ambient temperature and OST result in decreasing the water molecule energy at the ocular surface, thus fewer molecules will be able to leave the ocular surface, which leads to a decrease in the evaporation rate.²⁶ Also, water-holding capacity of the atmosphere decreases with decreasing ambient temperature, therefore the atmosphere can hold very little moisture.²⁷ These two factors working together may explain the reduction in evaporation rate in cold conditions and the negative relationship observed between lipid layer thickness and evaporation rate at low temperatures.

This study shows significant changes in tear stability at low temperatures. The mean NITBUT value at 5°C was 7.31 seconds. It is accepted that tear film with a break-up time of less than 10 seconds is considered an unstable tear film (moderate dry eye – grade 2).¹ Moreover, recent work has demonstrated that the mean interblink interval (IBI) is 7.5 seconds.²⁸ In the current work, a NITBUT value of less than the typical IBI was recorded at 10 and 5°C, that may result in possible repeated exposure of the ocular surface. Consequently, frequent ocular surface exposure could lead to the development of the signs and symptoms of dry eye and visual disturbances. Previous studies have shown that tear stability is changed as tear film exposed to different ambient temperature.⁸ Several studies have revealed that tear stability is the function of lipid layer integrity.²⁹⁻³² A change in the tear film lipid layer in a cold environment described earlier could be the reason behind the instability of tear film noted in this study.

In this study, it was found that ocular surface temperature decreased significantly as the ambient temperature decreased in 5°C steps. Pairwise comparison showed a significant difference in OST in all environmental conditions with the exception of that between 15 and 20°C ($p=0.093$). In the current study the mean change in central ocular surface temperature

was $0.18 \pm 0.40^\circ\text{C}$ for each 1°C change in room temperature. A drop between 0.15 and 0.21°C in human ocular surface temperature for each 1°C reduction in ambient temperature has been reported.^{33,34} It has been suggested that a decrease in ambient temperature could result in significant reduction in corneal metabolic activity, which could be the reason behind the reduction in corneal temperature.³⁵ However, it should be considered that metabolic rate may be not affected immediately with decreasing ambient temperature, therefore immediate change in OST could be resulted from another factor such as increasing evaporation rate.

Conclusion

This study has shown that ambient temperature influences tear film parameters. The current findings add to our understanding of the inter-relationship between tear film parameters and the physiological linkage between these parameters. The stability of tear film and lipid layer thickness were adversely affected at room temperatures of less than 10°C . NITBUT of less than the suggested IBI was recorded which may lead to exposure and to ocular surface pathology and a visual disturbance.

The evaporation rate showed a significant increase when the tear film was exposed to a room temperature of more than 20°C , and values of higher than the cut-off value of normal tear film were observed at a room temperature of 25°C .

Therefore, the effect of low or high ambient temperature on the tear film should not be neglected by people who work outdoors and spend a long time in adverse climate conditions or individual who play winter sports such as skiing and ice skating. In the same way, indoor workplaces need to be able to be maintained at a healthy and comfortable temperature range to ensure ocular comfort and well-being.

211 **References**

- 212 1. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye
213 disease: Report of the Definition and Classification Subcommittee of the international Dry
214 Eye WorkShop Ocul Surf 2007;5:75-92.
- 215 2. Gonzalez-Garcia MJ, Gonzalez-Saiz A, de la Fuente B, et al. Exposure to a controlled
216 adverse environment impairs the ocular surface of subjects with minimally symptomatic dry
217 eye. Invest Ophthalmol Vis Sci 2007;48:4026-32.
- 218 3. Sato M, Fukayo S, Yano E. Adverse environmental health effects of ultra-low relative
219 humidity indoor air. J Occup Health 2003;45:133-6.
- 220 4. Norback D, Lindgren T, Wieslander G. Changes in ocular and nasal signs and
221 symptoms among air crew in relation to air humidification on intercontinental flights.
222 Scandinavian Journal of Work Environment & Health 2006;32:138-44.
- 223 5. Reinikainen L, Jaakkola J. Significance of humidity and temperature on skin and
224 upper airway symptoms. Indoor Air 2003;13:344-52.
- 225 6. de Magalhaes Rios JL, Boechat JL, Gioda A, dos Santos CY, de Aquino Neto FR,
226 Lapa e Silva JR. Symptoms prevalence among office workers of a sealed versus a non-sealed
227 building: Associations to indoor air quality. Environ Int 2009;35:1136-41.
- 228 7. Wolkoff P. "Healthy" eye in office-like environments. Environ Int 2008;34:1204-14.
- 229 8. Paschides CA, Stefaniotou M, Papageorgiou J, Skourtis P, Psilas K. Ocular surface
230 and environmental changes. Acta Ophthalmol Scand 1998;76:74-7.
- 231 9. Borchman D, Foulks GN, Yappert MC, Mathews J, Leake K, Bell J. Factors
232 Affecting Evaporation Rates of Tear Film Components Measured In Vitro. Eye Contact
233 Lens-Sci Clin Pra 2009;35:32-7.
- 234 10. Horven I. Corneal temperature in normal subjects and arterial occlusive disease Acta
235 Ophthalmol (Copenh) 1975;53:863-74.
- 236 11. Hata S, Sakata M, Watanabe A, Fujishima H, Tsubota K. Corneal temperature and
237 inter-blinking time. Invest Ophthalmol Vis Sci 1994;35:S999.
- 238 12. Girardin F, Orgul S, Erb C, Flammer J. Relationship between corneal temperature and
239 finger temperature. Arch Ophthalmol 1999;117:166-9.
- 240 13. Rysa P, Sarvaran J. Thermography of the eye during cold stress. Acta Ophthalmol
241 (Copenh) 1974;123:234-9.
- 242 14. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and
243 validity of the ocular surface disease index. Arch Ophthalmol 2000;118:615-21.
- 244 15. Hirji N, Patel S, Callander M. Human tear film pre-rupture phase time (TP-RPT)- A
245 non-invasive technique for evaluation the pre-corneal tear film using a novel keratometer
246 mire. Ophthalmic Physiol Opt 1989;9:139-42.
- 247 16. Abusharha AA, Pearce EI. The Effect of Low Humidity on the Human Tear Film.
248 Cornea 2012;32:429-34.
- 249 17. Purslow C, Wolffsohn J. The relation between physical properties of the anterior eye
250 and ocular surface temperature. Optom Vis Sci 2007;84:197-201.
- 251 18. Guillon JP. Non-invasive Tearscope Plus routine for contact lens fitting. Cont Lens
252 Anterior Eye 1998;21 Suppl 1:S31-40.
- 253 19. Trees GR, Tomlinson A. Effect of Artificial Tear Solutions and Saline on Tear Film
254 Evaporation. Optom Vis Sci 1990;67:886-90.
- 255 20. Borchman D, Foulks GN, Yappert MC, Ho DV. Temperature-induced conformational
256 changes in human tear lipids hydrocarbon chains. Biopolymers 2007;87:124-33.

21. Nagymihalyi A, Dikstein S, Tiffany JM. The influence of eyelid temperature on the delivery of meibomian oil. *Exp Eye Res* 2004;78:367-70.
22. Butovich IA. On the lipid composition of human meibum and tears: comparative analysis of nonpolar lipids. *Invest Ophthalmol Vis Sci* 2008;49:3779-89.
23. Brown SI, Dervichian D. The oils of the meibomian glands: physical and surface characteristics. *Arch Ophthalmol* 1969;82:537.
24. Butovich IA, Arciniega JC, Wojtowicz JC. Meibomian lipid films and the impact of temperature. *Invest Ophthalmol Vis Sci* 2010;51:5508-18.
25. Tomlinson A, Doane MG, McFadyen A. Inputs and Outputs of the Lacrimal System: Review of Production and Evaporative Loss. *Ocul Surf* 2009;7:186-98.
26. Garai J. Physical model for vaporization. *Fluid Phase Equilib* 2009;283:89-92.
27. Gessert G. Measuring a medium's air space and water holding capacity. *Ornamentals Northwest* 1976;3:59-60.
28. Ousler GW, Hagberg KW, Schindelar M, Welch D, Abelson MB. The Ocular Protection Index. *Cornea* 2008;27:509-13.
29. Blackie CA, Solomon JD, Scaffidi RC, Greiner JV, Lemp MA, Korb DR. The Relationship Between Dry Eye Symptoms and Lipid Layer Thickness. *Cornea* 2009;28:789-94.
30. Isreb MA, Greiner JV, Korb DR, et al. Correlation of lipid layer thickness measurements with fluorescein tear film break-up time and Schirmer's test. *Eye* 2003;17:79-83.
31. Foulks GN. The correlation between the tear film lipid layer and dry eye disease. *Surv Ophthalmol* 2007;52:369-74.
32. Foulks GN, Bron AJ. Meibomian gland dysfunction: a clinical scheme for description, diagnosis, classification, and grading. *Ocul Surf* 2003;1:107-26.
33. Mapstone R. Determinants of corneal temperature. *Br J Ophthalmol* 1968;52:729-41.
34. Morgan PB. Ocular Thermography in Health and Disease [Ph.D. thesis]. Manchester, University of Manchester 1994.
35. Freeman R, Fatt I. Environmental influences on ocular temperature. *Invest Ophthalmol Vis Sci* 1973;12:596-602.

289 **Figures legend**

290 **Figure 1.** Comparison of the distribution of lipid layers pattern over range of ambient
291 temperature. Thicker lipid pattern was observed in higher room temperature (A). A box plot
292 showing tear evaporation rate (B) measured at 5, 10, 15, 20 and 25 °C. Significant increase in
293 tear evaporation was found as ambient temperature was increased. NITBUT (C) became
294 shorter when tear film was exposed to lower temperature. The tear break up time at 5°C was
295 significantly shorter than all other room temperatures. Significant difference was seen in
296 ocular surface temperature (D) between all ambient temperatures with exception of that
297 between 15 and 20°C ($n=12$, $p=0.093$). The box represents the interquartile range that
298 contains 50% of the values. The whiskers are lines that extend from the box to the highest
299 and lowest values, excluding outliers (O) that are 1.5 to 3 box lengths from the upper and
300 lower edge of the box and extremes (*) that are more than 3 box lengths. The line across the
301 box indicates the median value. Pairwise significant differences are shown (*)

302 **Figure 2.** Scatter plot showing correlation between ocular surface temperature and
303 evaporation rate (A), evaporation rate and tear break up time (B), evaporation rate and lipid
304 layer thickness (C), tear break up time and lipid layer thickness(D).

305

Figure 1. Comparison of the distribution of lipid layers pattern over range of ambient temperature. Thicker lipid pattern was observed in higher room temperature (A). A box plot showing tear evaporation rate (B) measured at 5, 10, 15, 20 and 25 °C. Significant increase in tear evaporation was found as ambient temperature was increased. NITBUT (C) became shorter when tear film was exposed to lower temperature. The tear break up time at 5°C was significantly shorter than all other room temperatures. Significant difference was seen in ocular surface temperature (D) between all ambient temperatures with exception of that between 15 and 20°C ($n=12$, $p=0.093$). The box represents the interquartile range that contains 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers (O) that are 1.5 to 3 box lengths from the upper and lower edge of the box and extremes (*) that are more than 3 box lengths. The line across the box indicates the median value. Pairwise significant differences are shown (*)

Figure 2. Scatter plot showing correlation between ocular surface temperature and evaporation rate (A), evaporation rate and tear break up time (B), evaporation rate and lipid layer thickness (C), tear break up time and lipid layer thickness (D).