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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

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1 **Effect of ambient temperature on the human tear film**

2 **Abstract**

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

6 **Method:** A Controlled Environment Chamber (CEC) was used to create different ambient
7 temperatures (5°C, 10°C, 15°C, 20°C, 25°C) at a constant relative humidity of 40%. Subjects
8 attended for two separate visits and were exposed to 25°C, 20°C and 15°C at one visit and to
9 10°C and 5°C at the other visit. The subjects were exposed to each room temperature for 10
10 minutes before investigating tear film parameters. The order of the visits was random. Tear
11 physiology parameters assessed were tear evaporation rate, non-invasive tear breakup time
12 (NIBUT), lipid layer thickness (LLT), and ocular surface temperature (OST). Each parameter
13 was assessed under each condition.

14 **Result:** A three-fold increase in tear evaporation rate was observed as ambient temperature
15 increased to 25 °C ($p=0.00$). The mean evaporation rate increased from 0.056 $\mu\text{l}/\text{min}$ at 5°C,
16 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
17 25°C ($p= 0.01$). A significant change in LLT was also observed ($p= 0.00$), LLT median
18 ranged between to 20 to 40nm at 5 and 10 °C and increased to 40 and 90nm at 15, 20 and
19 25°C . Mean reduction of 4°C OST was observed as ambient temperature decreased from 25
20 to 5°C.

21 **Conclusion:** Ambient temperature has a considerable effect on human tear film
22 characteristics. Tear evaporation rate, tear lipid layer thickness, tear stability and ocular
23 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

27 **Introduction**

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

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

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

54 **Method**

55 Ethical approval was obtained from the Glasgow Caledonian University Human Ethics
56 Committee. Subjects were healthy normals with no evidence of dry eye. Inclusion criteria
57 was Ocular Surface Disease Index (OSDI) ¹⁴ score of less than 12 and NITBUT of more than
58 10 seconds using HIRCAL grid.¹⁵ Twelve healthy normal subjects (3 female, 9 male, mean \pm
59 Sd 29.4 \pm 2.4 years) with no current ocular diseases or surgery were enrolled in this study.

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

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

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

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

91 Variables were tested for normality using a Kolmogorov-Smirnov test. A repeated measure
92 ANOVA and Tukey's post-hoc test were applied for normally distributed data while the
93 ordinal and data with non-normal distribution were analysed using Friedman's test and post-
94 hoc Wilcoxon rank-sum test. Correlation between tear parameters was assessed using
95 Pearson's test and Spearman's test for the data with normal and non-normal distribution
96 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

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

121 **Ocular surface temperature**

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

126 Correlation tests were applied to determine the relationship between tear parameters as
127 temperature was altered. Data from all temperatures were combined and analyzed using
128 either Persons's (R) for normally distributed data or Spearman's (ρ) correlation tests for
129 ordinal and data with non-normal distribution. In Figure 2A a scatter plot shows a positive
130 relationship between ocular surface temperature and evaporation rate ($r = 0.45, p<0.05$).
131 Also, a significant correlation noted between evaporation rate and tear break-up time ($r =$
132 $0.32, p<0.001$ (Figure 2B). Tear evaporation was also showed a negative relationship with
133 lipid layer thickness ($\rho = 0.43, p<0.001$) (Figure 2C). Also a significant correlation
134 between lipid layer thickness and tear film stability was found ($\rho= 0.49, p <0.001$) (Figure
135 2D).

136 **Discussion**

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

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

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

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

158 A change in tear evaporation rate was observed when the ambient temperature changed. The
159 evaporation rate increased as the temperature increased. The mean evaporation rate at 25°C
160 was double that observed at 10°C, and triple the values recorded at 5°C. Box plots of
161 evaporation rate at 25°C show that a quarter of participants had an evaporation rate
162 characteristic of dry eye patients (evap>0.23µl/min²⁵). The lipid layer plays a critical role in
163 controlling tear film evaporation. However, although a thin lipid layer was observed at 5 and
164 10°C, the evaporation rate was low in the cold environment, meaning that there may be

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

173 This study shows significant changes in tear stability at low temperatures. The mean
174 NITBUT value at 5°C was 7.31 seconds. It is accepted that tear film with a break-up time of
175 less than 10 seconds is considered an unstable tear film (moderate dry eye – grade 2).¹
176 Moreover, recent work has demonstrated that the mean interblink interval (IBI) is 7.5
177 seconds.²⁸ In the current work, a NITBUT value of less than the typical IBI was recorded at
178 10 and 5°C, that may result in possible repeated exposure of the ocular surface. Consequently,
179 frequent ocular surface exposure could lead to the development of the signs and symptoms of
180 dry eye and visual disturbances. Previous studies have shown that tear stability is changed as
181 tear film exposed to different ambient temperature.⁸ Several studies have revealed that tear
182 stability is the function of lipid layer integrity.²⁹⁻³² A change in the tear film lipid layer in a
183 cold environment described earlier could be the reason behind the instability of tear film
184 noted in this study.

185 In this study, it was found that ocular surface temperature decreased significantly as the
186 ambient temperature decreased in 5°C steps. Pairwise comparison showed a significant
187 difference in OST in all environmental conditions with the exception of that between 15 and
188 20°C ($p=0.093$). In the current study the mean change in central ocular surface temperature

189 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
190 in human ocular surface temperature for each 1°C reduction in ambient temperature has been
191 reported.^{33,34} It has been suggested that a decrease in ambient temperature could result in
192 significant reduction in corneal metabolic activity, which could be the reason behind the
193 reduction in corneal temperature.³⁵ However, it should be considered that metabolic rate may
194 be not affected immediately with decreasing ambient temperature, therefore immediate
195 change in OST could be resulted from another factor such as increasing evaporation rate.

196 **Conclusion**

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

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

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

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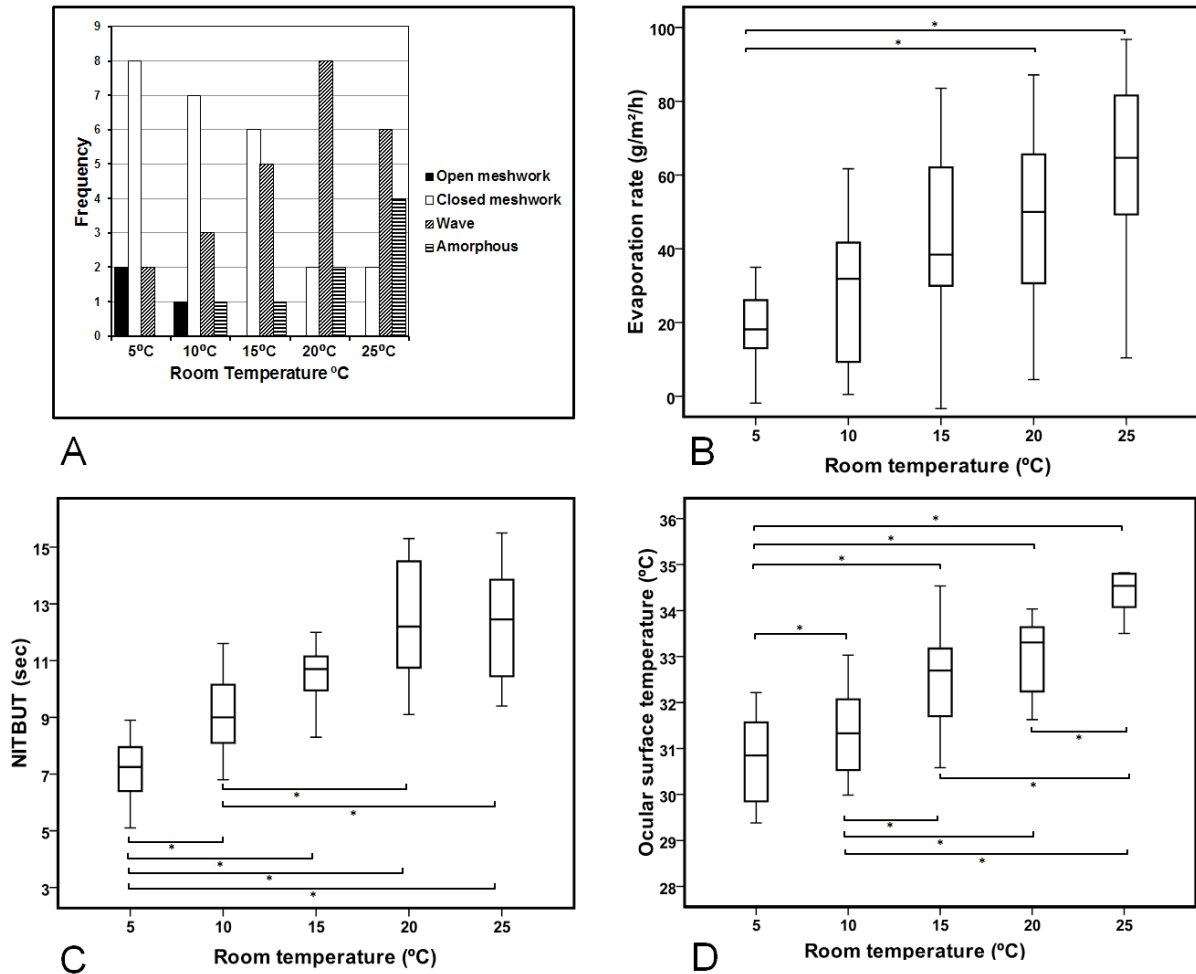
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289 **Figures legend**

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).

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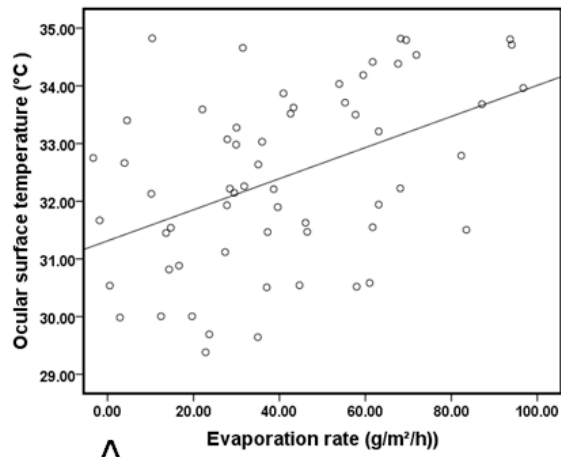
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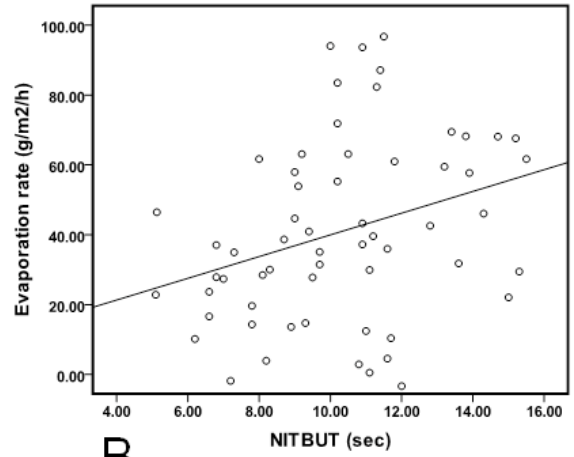
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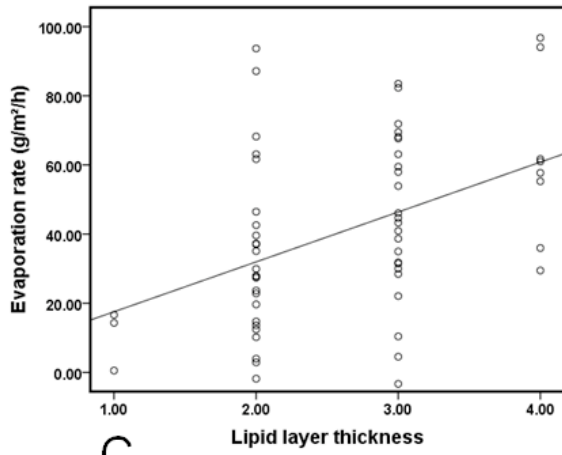
Figure 1. Comparison of the distribution of lipid layers pattern over range of ambient temperature. Thicker lipid pattern was observed in higher room temperature (A). A box plot showing tear evaporation rate (B) measured at 5, 10, 15, 20 and 25 °C. Significant increase in tear evaporation was found as 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 (*)



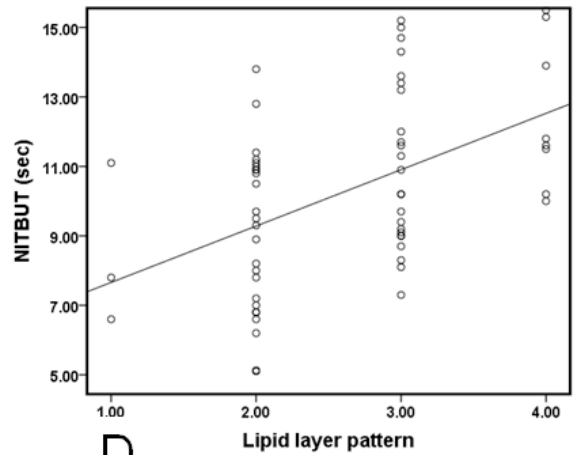
A



B



C



D

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

323