Journal of Human Kinetics volume 67/2019, 143-151 DOI: 10.2478/hukin-2019-0004
**Section II - Exercise Physiology & Sports Medicine**


sciendo

# Alteration of Blinking and Sex Differences During Physical Exercise Affect Tear Osmolarity

*by*
*Adam Wylęgała[1], Bogumiła Sędziak-Marcinek[1], Jan Pilch[2], Edward Wylęgała[1]*

*Physical exertion leads to the rise in tear osmolarity. However, previous studies have been conducted mostly on males and did not consider sex differences and the possible alteration in blinking during physical exercise. Sixteen women and 18 men aged 25.09 ± 1.70 were divided into equal groups with eyes open and shut. Participants performed 8-min medium-intensity exercise and 5-min intense exercise on a cycloergometer. Tear osmolarity (in mOsm/L) was evaluated before (T0), after medium-intensity (T1) and intense exercise (T2). The blinking rate was assessed in a group with eyes open. Tear brake up time was measured in T0 and T1. With tear osmolarity measuring 305.72 ± 1.22 and 313.56 ± 1.90 for men and women, respectively, we observed significant differences in T1. In T2, tear osmolarity in men was 303.3 ± 1.28 vs. 310.87 ± 1.36 in women. The blinking rate decreased from 14.24 ± 2.54/min in T0 to 9.41 ± 2.83/min in T1. There was a statistically significant change in tear osmolarity in both groups, that is, in the group with eyes shut from 300.53 ± 1.37 in T0 to 308.06 ± 1.55 in T1 to 304.88 ± 1.54 in T2. In the group with eyes open, tear osmolarity increased from 300.29 ± 1.37 in T0 to 310.76 ± 1.55 in T1 and then dropped to 308.88 ± 1.54 in T2. Tear brake up time measured in T0 was 14.7 ± 1.43 vs. 13.53 ±1.48 in the open eyes condition. Due to physical exercise, short-term changes in tear osmolarity are partially caused by altered blinking. Sex differences in tear osmolarity in response to exertion may confirm the relationship between total body water and tear osmolarity.*

***Key words***: *tearing, body water, sport, lacrimation, TearLab, Tosm.*

## Introduction

Dry eye disorder (DED) is one of the most common ophthalmic pathology, affecting many patients around the world. DED is a multifactorial, multisymptom disorder of the ocular surface, characterized by tear film instability, hyperosmolarity, and inflammation (Nelson et al., 2017).

The knowledge about effects of physical exercise on ocular physiology is well documented. Its general effects on intra-ocular pressure (IOP), retinal and choroidal blood flow, myopia and retinal electrical function, have been studied extensively (Wylęgała, 2016). Tears are secretion of lacrimal glands composed of mainly water, protein, and electrolyte with osmolarity similar to plasma (Stahl et al., 2012; Tiffany, 2003). Risk factors for developing DED are a sedentary lifestyle and a low level of physical fitness. Currently, the only objective measurable variable of DED is tear osmolarity (Tosm) (Nelson et al., 2017). Measuring Tosm is the most accurate method for the DED diagnosis and follow-up (Benelli et al., 2010; Farris, 1994). Previously, a larger amount of tears were required to be collected to measure Tosm, and the process of collection might have led to reflex lacrimation; moreover, the long time needed for this procedure might have been a source of large variability. Currently, it is possible to conduct Tosm measurements within a minute using a TearLab

[1] - *Ophthalmology Department, Railway Hospital, Katowice Panewnicka 65. 40765- Katowice, Poland. II School of Medicine with the Division of Dentistry in Zabrze Medical University of Silesia, Katowice, Poland.*
[2] - *Chair of Physiological-Medical Sciences. The Jerzy Kukuczka Academy of Physical Education in Katowice ul. Mikołowska 72a, 40-065 Katowice, Poland.*

*Authors submitted their contribution to the article to the editorial board.*
*Accepted for printing in the Journal of Human Kinetics vol. 67/2019 in June 2019.*

device (Sollanek et al., 2012). In their study, Vera et al. (2017) demonstrated that physical exertion led to the rise in Tosm in untrained patients 5 min after strenuous exercise compared to the trained group. Yet, the authors did not show whether such changes were attributed to the difference in the blinking rate or the rise of the evaporation rate due to the increase in body temperature. In previous studies, the effects of physical exercise on Tosm were evaluated mostly on males with eyes open (Fortes et al., 2011; Ungaro et al., 2015; Vera et al., 2017), thus further research is required with groups that would include more female participants. Eye blinking is related to specific condition; its rate decreases during tasks that require attention (Stern et al., 1984). Based on the observation in other papers, we thought that some of the changes in Tosm might be the result of the decreased blinking rate. Therefore, the aim of the present study was to investigate the effects of blinking and sex on Tosm during physical exercise.

## Methods

***Participants***

The study was conducted in 2017 and 2018 on 34 voluntary 5th-year medical students (18 men and 16 women). Table 1 shows the detailed biometrical results.

All participants gave their written informed consent and received a leaflet describing the procedures before the study, which received approval from the Ethics Committee. The study followed the tenets of the Declaration of Helsinki. Participants filled out the Polish version of the ocular surface disease index questionnaire and underwent full ophthalmic examinations one day before the study commenced. DED patients, contact lens wearers, and patients currently receiving any topical ocular medications, as well as participants with contraindicated systemic conditions, were excluded from the study. Only participants with a medium level of fitness defined as a weekly physical activity from 1.5 to 4 hours were enrolled in the study.

Measures

The study was conducted in constant temperature of 22°C and 40% humidity, in the morning between 10-12 am. Temperature and humidity was measured with a thermo-hygrometer (Beurer HM 16, Beurer GmbH, Ulm, Germany). The participants were seated at least 30 min in the study room before the beginning of the experiment.

Participants were divided into two equal groups. In the study group, participants during the exercise were recorded with iPhone 6 camera (Apple, Cupertino, USA), while the control group had the eyes shut. Using an open software code available at (https://www.pyimagesearch.com/2017/04/24/eye-blink-detection-opencv-python-dlib/), the blinking rate was calculated. The blinking rate was then calculated again by dividing the number of blinks per minute. Tosm was measured using TearLab (TearLab Osmolarity System, TearLab Corp., San Diego, CA). It is designed to take a 50 nL sample of tears without creating reflex tearing. According to the user manual, this device was calibrated each day with the control 334 mmol/L card before measurements. Furthermore, two random cards were also verified with provided osmolarity control solutions after opening a new box of 42 cards. Tear film brake-up time (Tbut) was calculated in T0 and T2 using OCULUS Keratograph® 5M (Oculus, Wetzlar, Germany).

Using a patient monitor FX 3000 (Emtel, Zabrze, Poland), the HR, blood pressure and blood oxygen saturation were measured. The workload was calculated individually based on the following formula: $HR_{max}$: 220-age, %Heart rate capacity(HRC)=100×(HR after exercise-HR sitting)/($HR_{max}$-HR sitting) (Kinoshita et al., 2016).

***Design and Procedures***

Before the exercise, the HR, blood pressure and Tosm were evaluated. In a group with eyes open the blinking rate was measured 5 min before the exercise. Then the exercise started. Participants performed an incremental test on a cycloergometer BH Spada **(**Beistegui Hermanos S.A, Vitoria-Gasteiz, Spain). After the 3-min warm-up at the HR of 20-30% HRC, the minimal HR on the cycloergometer was set to 40-50% of the HRC for 5 min, and after this duration, Tosm and HR were measured (T1). Then, the HR was set up to 65-70% of HRC, and after 5 min of strenuous exercise (T2), Tosm was measured again together with the HR and systolic pressure.

***Statistical analysis***

Table 1 presents descriptive biometric variables that are unweighted means ± standard error of the mean or standard deviation obtained

with one-way ANOVA. Repeated measures ANOVA was used to investigate the effect of blinking on Tosm, with a post-hoc Bonferroni test. Using factorial ANOVA with the Bonferroni post-hoc test, sex differences and blinking were calculated. All statistical tests were checked for equality of variance with the Levene's test and the Shapiro–Wilk test for normality. Correlation tests were followed by one-way ANOVA. Statistical significance of $p \leq 0.05$ was accepted; Statistica v10.2.1 (Statsoft, Cracow, Poland) was used in all calculations.

## Results

There were statistically significant increases in Tosm in both groups, in the group with eyes shut from 300.53 ± 1.37 mOsm/L in T0 to 308.06 ± 1.55 mOsm/L ($p < 0.0001$) in T1 that later dropped to 304.88 ± 1.54 mOsm/L in T2 ($p = 0.007$) compared to the control group. There were statistically significant increases in the group with eyes open between 300.29 ± 1.37 mOsm/L in T0 and 310.76 ± 1.55 mOsm/L ($p < 0.0001$) in T1 and then this value dropped to 308.88 ± 1.54 mOsm/L ($p < 0.001$). The changes between T1 and T2 in the control and study groups were not significant.

*Sex differences*: Men had slightly lower Tosm of 299.94 ± 1.33 mOsm/L compared to women (300.94 ± 1.41 mOsm/L). Tosm was 305.72 ± 1.22 mOsm/L and 313.56 ± 1.90 mOsm/L for men and women, respectively, in T1. Tosm in men was 303.3 ± 1.28 mOsm/L vs. 310.87 ± 1.36 mOsm/L in women in T2. Changes between men and women were significant in T1 ($p < 0.0001$) and T2 ($p < 0.001$).

*Blinking rate*: There was a statistically significant decrease in the blinking rate from 14.24 ± 2.54/min to 9.41 ± 2.83/min between 5 min before T0 and T0-T1 ($p < 0.001$). The blinking rate increased to 14.29 ± 1.83/min in the final phase of the experiment (Figure 3). Men presented a higher blinking rate than women before the exercises 14.7 ± 0.97/min vs. 13.9 ± 0.817/min. Between the start of the exercise and the first measurement (T1), the blinking rate decreased to 10.14 ± 1.077 and 8.9 ± 0.70/min in men and women, respectively. Between T1 and T2, blinking was 15.29 ± 0.63/min in men and 13.60 ± 0.53/min in women.

**Table 1**

*Biometric variables assessed before and during the experiment. Data provided as means ± standard error (M ± SE).*

| | | |
|---|---|---|
| Age | 25.26 ± 1.74 | 24.87 ± 1.63 |
| Sex | Male (n = 18) | Female (n = 16) |
| Age All Groups | 25.09 ± 1.70 | |
| BMI | 19.99 ± 1.18 | 20.74 ± 1.50 |
| BMI All Groups | 20.34 ± 1.37 | |
| Systolic pressure T0 | 136.33 ± 3.26 | 123.81 ± 3.46 |
| Diastolic pressure T0 | 76.39 ± 2.67 | 72.44 ± 2.84 |
| Systolic pressure T2 | 149.28 ± 3.50 | 140.00 ± 3.71 |
| Diastolic pressure T2 | 81.00 ± 2.18 | 77.25 ± 2.31 |
| HR T0 | 79.33 ± 3.47 | 81.44 ± 3.68 |
| HR T1 | 139.22 ± 3.95 | 137.56 ± 4.19 |
| HR T2 | 173.61 ± 4.69 | 166.69 ± 4.98 |

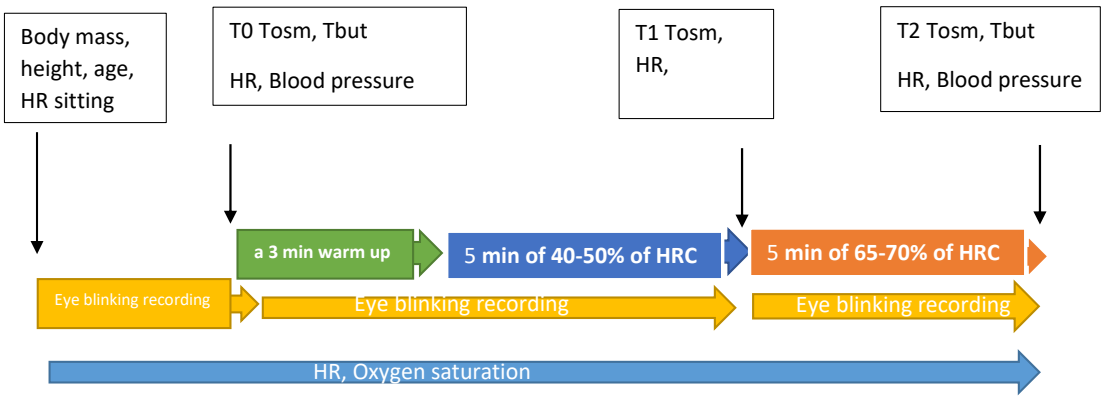


**Figure 1**

*The design of the study protocol. The same protocol was applied to both groups except for blinking recording in a group with eyes shut. The minimal HR was set to 20% of HRC during the warm up. The minimal HR was set to 40% and the maximal to 50% of the max HRC and 65 to 70% during T1 and T2, respectively. Participants' blood oxygen saturation and HR were monitored during all phases of the experiment.*

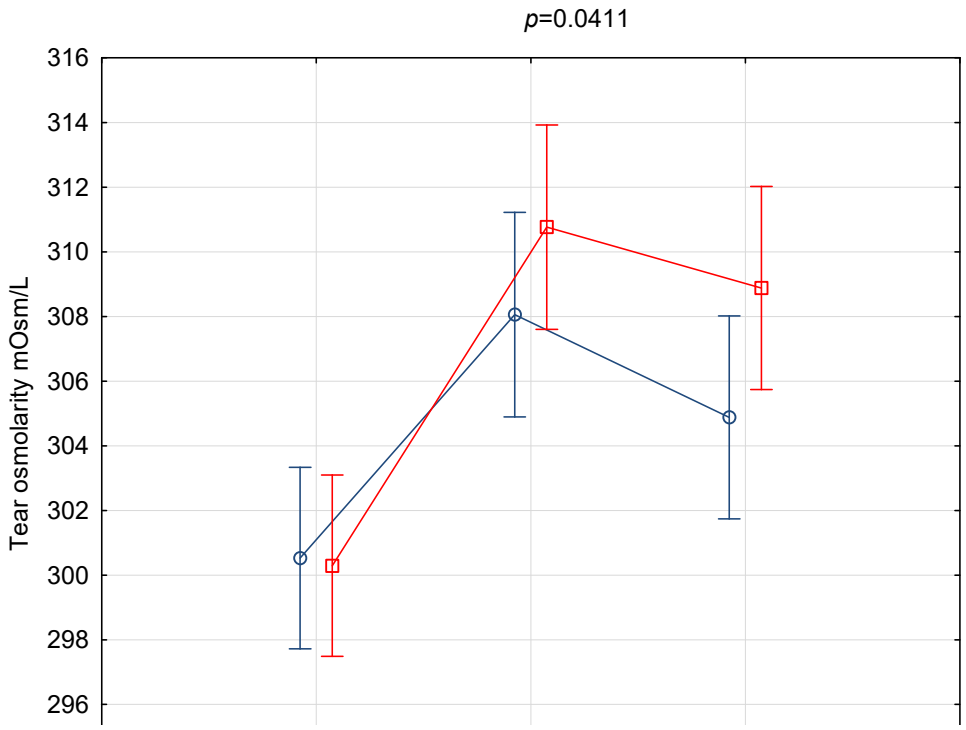


**Figure 2**

*Tosm changes in groups with eyes open and shut. Tosm was measured in T0, T1 and T2.*

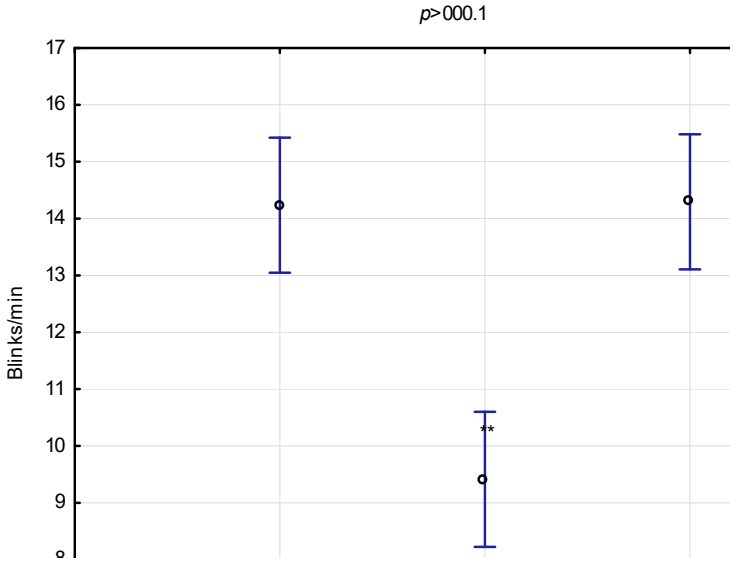


**Figure 3**

*The blinking rate per minute measured 5 minutes before T0, between T0 and T1 and between T1 and T2*

*** p < 0.001 T1 compared to the control group*

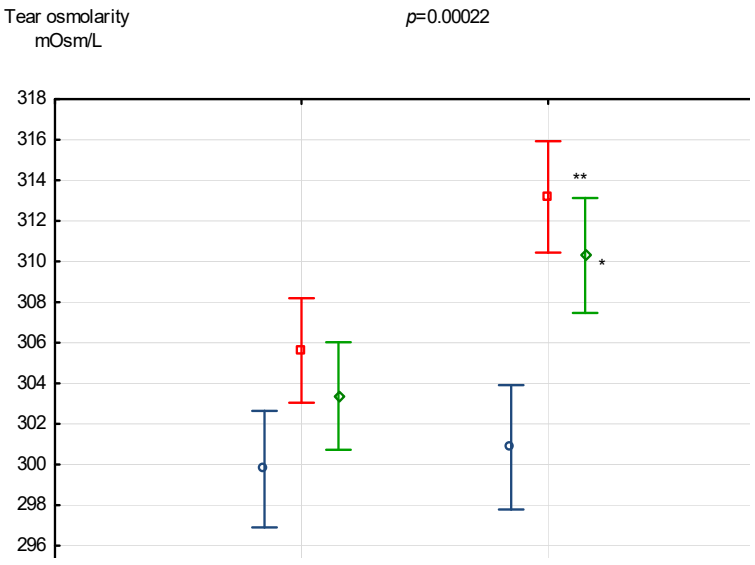


**Figure 4**

*Changes in osmolarity in male and female participants. Statistical significance was assessed between both groups at the same time points.*

*p > 0.001 ** p < 0.0001*

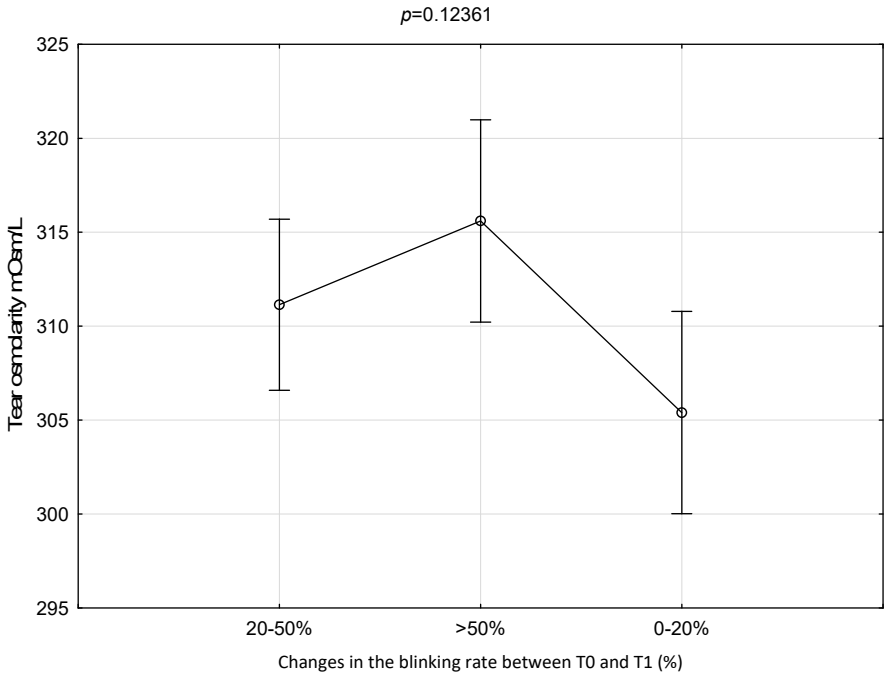


**Figure 5**

*Tosm changes correlated with blinking rate decreases.*

*Correlation between decreases in blinking and Tosm*: We tested whether the changes in the blinking rate correlated with the rise in Tosm. In the group with 0-20% decreases in the blinking rate, mean Tosm was 305.4 ± 2.5 mOsm/L; in the group with decreases between 20 and 50%, mean Tosm was 311.14 ± 2.12; in the group with decreases of more than 50%, the mean observed was 315.6 ± 2.51. We could not perform correlation analysis due to $p = 0.12361$ in one-way ANOVA.

*Tbut*: Tbut measured in T0 was 14.7 ± 1.43 vs. 13.53 ±1.48 in the open eyes condition, while in the group with eyes shut it was 14.3 ± 0.9 and 14.54 ± 0.84. The results were not significant ($p = 0.64$). The mean Tbut measured in T0 in males was 14.56 ± 1.39 vs. 14.40 ± 0.81 in females, while in T2 it was 14.36 ± 1.05 and 13.68 ± 1.35; these results were non-significant.

## Discussion

In our study, we aimed to observe sex changes in Tosm during physical exercise and how the blinking affected Tosm. We indicated that the changes in Tosm were different in men and women, in response to physical exercise and that changes in Tosm were partially attributed to alterations in the blinking rate. Previous studies have shown some correlation between hydration status and IOP, as well as Tosm and tear breakup time (Vera et al., 2017; Sollanek et al., 2012). Moreover, some authors have found a positive correlation between body fat and the occurrence of DED (Ho et al., 2017). Physical exercise leads to body water deficit caused by increased breathing, diuresis, and sweating (Zwierko et al., 2015). Vera et al. (2017) concluded that a higher fitness level eliminated the increase that the exercise had on

Tosm in male military pilots.They compared the effects of a maximal incremental test on trained and untrained participants. They observed a 12.33 mmol/L increase in Tosm in the untrained group, while it remained stable (~1.45 mmol/L) in the trained one. The authors measured Tosm 5 min post maximal exercise (Vera et al., 2017). The fitness level and the amount of adipose tissue have been inversely linked to the prevalence of DED (Ho et al., 2017). Physical exercise leads to an expansion of plasma and blood volume. Hypervolemia is then caused by the increased amount of blood flowing through the muscles and skin. Water is further lost for thermoregulation by sweating and exhaling (Convertino, 2007). Such changes in water distribution can consequently decrease the amount of secreted tears. Furthermore, strenuous exercise can lead to a decrease in the ocular blood flow (Ikemura and Hayashi, 2014). It is known that adult men have more lean tissue than women (Taylor et al., 2010), while adult women have less fat-free mass and total body water compared to adult men (Kotler et al., 1996). This might explain the higher rise of Tosm in women than men as an increase in blood and plasma volume during an exercise leads to more rapid changes in tissue water distribution. Fortes et al. (2011) showed that Tosm increased during exercise and corelated with plasma osmolarity. During exertion in heat with fluid restriction, Tosm changed from 293 ± 9 to 305 ± 13 mOsm/L. Tosm values were restored in the next day morning or after hydration. The authors linked hydration status of the body with Tosm (Fortes et al., 2011), yet, the study included 10 men and 4 women only. Similar correlations between Tosm and the hydration rate were drawn by Ungaro et al. (2015). Hooland et al. (2017) presented that Tosm increased up to 8 ± 15 mOsm/L during a self-paced 10-km outdoor run. The increase correlated with decreases in body water. The authors did not advocate using Tosm as a measure of hydration due to low correlation with well-established variables such as plasma osmolarity and urine osmolarity (Holland et al., 2017). Firstly, measuring Tosm while running outdoors can, however, create some false results as runners do not necessarily present the same $\%HR_{max}$; secondly, outdoor condition can stimulate reflex tearing. TearLab is considered accurate and precise provided that it is calibrated before each day of testing (Keech et al., 2013; Sullivan et al., 2012; Yoon et al., 2014). Downie (2015) showed that shorter Tbut correlated with higher Tosm values in patients with DED. In our study Tosm was higher at the end of the experiment and Tbut was shorter than at the beginning.

A spontaneous eye-blink rate shows a diurnal variation with the highest rate in the evening (Barbato et al., 2000). There are three types of blinking as follows: spontaneous, reflex, and voluntary. Spontaneous blinking is affected by mental and cognitive processes. People blink spontaneously around 12 times/min, but this can drop to 4.5 times/min during reading (Bentivoglio et al., 1997). In our study, we also observed similar blinking rates. It is widely accepted that the blinking rate diminishes during tasks that require visual coordination, such as sport activity (Drew, 1951). In our study, we showed changes in Tosm during exercise that were irrespective of the status of the eyelids. This means that the changes were not only the result of the heat-induced evaporation. According to our knowledge, this is the first study that also measured the changes in Tosm during low- to medium-intensity exercise. We can hypothesize why the increases in Tosm were greater after 8 min of medium-intensity exercise than after 5 min of strenuous exercise. Part of this might be a result of a different metabolism during strenuous and mild exercise, and a rapid rise in nor- and epinephrine after the start of exercise (Horton et al., 1998). Some of the rise in Tosm may be a result of the rise in temperature in the first minutes of exercise (Lim et al., 2008). Future studies should focus more on changes in Tosm after mild exercises and be conducted with eyes shut. The current study also had some limitations. First, we performed our assessment on young healthy males and females free from DED; secondly, the students were sometimes looking at the display of the bicycle and this might have diminished their blinking rate. We did not measure the body temperature due to abundance of such studies. Moreover, we examined Tosm right after the cessation of the exercise as we were more interested in dynamic changes in Tosm during exertion. We also did not measure Tbut in T1 as this would require moving the participants and prolong the experiment.

## Acknowledgements

This work was supported by the Silesian Medical University under Grant KNW-1-164/N/6/0.

## References

Barbato G, Ficca G, Muscettola G, Fichele M, Beatrice M, Rinaldi F. Diurnal variation in spontaneous eye-blink rate. *Psychiatry Res*, 2000; 93: 145–151

Benelli U, Nardi M, Posarelli C, Albert TG. Tear osmolarity measurement using the TearLab™ Osmolarity System in the assessment of dry eye treatment effectiveness. *Contact Lens Anterior Eye*, 2010; 33: 61–67

Bentivoglio AR, Bressman SB, Cassetta E, Carretta D, Tonali P, Albanese A. Analysis of blink rate patterns in normal subjects. *Mov Disord*, 1997; 12: 1028–1034

Convertino VA. Blood Volume Response to Physical Activity and Inactivity. *Am J Med Sci*, 2007; 334: 72–79

Downie LE. Automated tear film surface quality breakup time as a novel clinical marker for tear hyperosmolarity in dry eye disease. *Investig Ophthalmol Vis Sci*, 2015; 56: 7260–7268

Drew GC. Variations in reflex blink-rate during visual-motor tasks. *Q J Exp Psychol*, 1951; 3: 73–88

Farris RL. Tear osmolarity--a new gold standard? *Adv Exp Med Biol*, 1994; 350: 495–503

Fortes MB, Diment BC, Felice U DI, Gunn AE, Kendall JL, Esmaeelpour M, Walsh NP. Tear Fluid Osmolarity as a Potential Marker of Hydration Status. *Med Sci Sport Exerc*, 2011; 43: 1590–1597

Ho KC, Jalbert I, Watt K, Golebiowski B. A Possible Association Between Dry Eye Symptoms and Body Fat. *Eye Contact Lens Sci Clin Pract*, 2017; 43: 245–252

Holland JJ, Ray M, Irwin C, Skinner TL, Leveritt M, Desbrow B. Tear osmolarity is sensitive to exercise-induced fluid loss but is not associated with common hydration measures in a field setting. *J Sports Sci*, 2017; 1–8

Horton TJ, Pagliassotti MJ, Hobbs K, Hill JO. Fuel metabolism in men and women during and after long-duration exercise. *J Appl Physiol*, 1998; 85: 1823–1832

Ikemura T, Hayashi N. Effects of heat stress on ocular blood flow during exhaustive exercise. *J Sports Sci Med*, 2014; 13: 172–179

Keech A, Senchyna M, Jones L. Impact of Time Between Collection and Collection Method on Human Tear Fluid Osmolarity. *Curr Eye Res*, 2013; 38: 428–436

Kinoshita T, Mori J, Okuda N, Imaizumi H, Iwasaki M, Shimizu M, Miyamoto H, Akaiwa K, Semba K, Sonoda S, Sakamoto T, Mitamura Y. Effects of Exercise on the Structure and Circulation of Choroid in Normal Eyes. *PLoS One*, 2016; 11(12):e0168336

Kotler DP, Burastero S, Wang J, Pierson RN. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex, and disease. *Am J Clin Nutr*, 1996; 64: 489S–497S

Lim CL, Byrne C, Lee JK. Human thermoregulation and measurement of body temperature in exercise and clinical settings. *Ann Acad Med Singapore*, 2008; 37: 347–353

Nelson JD, Craig JP, Akpek EK, Azar DT, Belmonte C, Bron AJ, Clayton JA, Dogru M, Dua HS, Foulks GN. TFOS DEWS II Introduction. *Ocul Surf*, 2017; 15: 269–275

Sollanek KJ, Kenefick RW, Walsh NP, Fortes MB, Esmaeelpour M, Cheuvront SN. Assessment of thermal dehydration using the human eye: What is the potential? *J Therm Biol*, 2012; 37: 111–117

Stahl U, Willcox M, Stapleton F. Osmolality and tear film dynamics. *Clin Exp Optom*, 2012; 95: 3–11

Stern JA, Walrath LC, Goldstein R. The Endogenous Eyeblink. *Psychophysiology*, 1984; 21: 22–33

Sullivan BD, Crews LA, Sönmez B, la Paz MF de, Comert E, Charoenrook V, Araujo AL de, Pepose JS, Berg MS, Kosheleff VP. Clinical Utility of Objective Tests for Dry Eye Disease. *Cornea*, 2012; 31: 1000–1008

Taylor RW, Grant AM, Williams SM, Goulding A. Sex Differences in Regional Body Fat Distribution From Pre- to Postpuberty. *Obesity*, 2010; 18: 1410–1416

Tiffany JM. Tears in health and disease. *Eye*, 2003; 17: 923–926

Ungaro CT, Reimel AJ, Nuccio RP, Barnes KA, Pahnke MD, Baker LB. Non-invasive estimation of hydration status changes through tear fluid osmolarity during exercise and post-exercise rehydration. *Eur J Appl Physiol*, 2015; 115: 1165–1175

Vera J, Jiménez R, Madinabeitia I, Masiulis N, Cárdenas D. A maximal incremental effort alters tear osmolarity depending on the fitness level in military helicopter pilots. *Ocul Surf*, 2017; 15: 795–801

Wylęgała A. The Effects of Physical Exercises on Ocular Physiology. *J Glaucoma*, 2016; 25: e843–e849

Yoon D, Gadaria-Rathod N, Oh C, Asbell PA. Precision and Accuracy of TearLab Osmometer in Measuring Osmolarity of Salt Solutions. *Curr Eye Res*, 2014; 39: 1247–1250

Zwierko T, Puchalska-Niedbał L, Krzepota J, Markiewicz M, Woźniak J, Lubiński W. The effects of sports vision training on binocular vision function in Female University Athletes. *J Hum Kinet,* 2015; 49: 287–296

**Corresponding author:**

**Adam Wylegala**
Ophthalmology Department Railway Hospital Katowice
Panewnicka 65. 40765- Katowice,
II School of Medicine with the Division of Dentistry in Zabrze Medical University of Silesia, Katowice
Tel. +48 32 6053592 fax +48 32 6053593
ORCID: 0000-0001-7295-4936
E-mail: adam.wylegala@gmail.com