Title: The effect of acute exercise on environmentally induced symptoms of dry-eye.

Running title: Exercise and dry-eye

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What is the central question of this study?

Does acute exercise attenuate or exacerbate symptoms of environmentally induced dry-eye?

What is the main finding and its importance?

Exposure to a desiccating environment increased the perception of dry-eye symptoms and concentration of matrix metalloproteinase 9 (MMP-9) in tears compared to a control environment. These findings were absent when participants completed an acute bout of exercise in the desiccating environment, suggesting that exercise may attenuate symptoms.

Abstract

The purpose of this study was to investigate the effects of acute exercise on environmentally induced symptoms of dry-eye. Twelve participants without dry-eye disease volunteered to complete three experimental visits in a randomised order; (1) control condition seated for 1-hour at a relative humidity (RH) of 40% (CONT), (2) dry condition seated for 1-hour at a RH of 20% (DRY), (3) exercise condition seated for 40-min followed by 20-min of cycling exercise at a RH of 20% (EXER). Tear volume, tear matrix metalloproteinase 9 (MMP-9), perception of dry-eye symptoms (frequency and severity), core temperature and ocular surface temperature (OST) were measured at the end of each exposure. The perception of dry-eye frequency and MMP-9 concentration were significantly higher in DRY compared to CONT ($P < 0.012$), with no differences in EXER compared to CONT. The results suggest that an acute bout of exercise may attenuate symptoms of environmentally induced dry-eye, and warrant further research.

Key words: Exercise, dry-eye, MMP-9, Schirmer, tears

Introduction

Chronic dryness of the ocular surface can manifest as dry-eye disease, a condition characterised by discomfort, inflammation, and blurring of vision between blinks (A. J. Bron et al., 2017). These symptoms can present acutely or chronically (Gayton, 2009; Schaumberg, Dana, Buring, & Sullivan, 2009) and the latter may lead to damage to the ocular surface. Acute presentation of dry-eye disease has been observed in people working (Sano, Kawashima, Takechi, Mimura, & Tsubota, 2018; Uchiyama, Aronowicz, Butovich, & McCulley, 2007) and exercising (Gayton, 2009; Qiao, 2012) in desiccating environments, during contact lens wear (Nichols & Sinnott, 2006), and in people working irregular shift patterns (Makateb & Torabifard, 2017). One hour of exposure to a relative humidity (RH) of 5% can induce dry-eye in otherwise non-sufferers (Abusharha & Pearce, 2013). The authors proposed that the thinner tear lipid layer observed in 5% RH may be influenced by alterations in protein structures on the corneal surface (Abusharha & Pearce, 2013). Matrix metalloproteinase 9 (MMP-9) is an endopeptidase involved with extracellular matrix remodelling and inflammation after corneal surface damage and has become a key biological marker for dry-eye due to its direct relationship with dry-eye severity (Kaufman, 2013). It is hypothesised that MMP-9 disrupts the corneal epithelial barrier facilitating ulceration and disrupting wound healing (Kaufman, 2013). Therefore, acute exposure of non-dry-eye sufferers to low RH conditions may provide a useful model to investigate interventions that may influence dry-eye, and MMP-9 appears to be a key objective variable of dry-eye induced ocular surface dysfunction.

Underexplored interventions that may influence dry-eye symptoms are exercise and physical activity. In a cross-sectional study, Kawashima et al. (2014) observed that participants without dry-eye disease tended to have higher levels of physical activity as measured by the International Physical Activity Questionnaire (IPAQ). It was postulated that reduced physical activity may exacerbate dry-eye by increasing the risk of chronic inflammatory diseases and oxidative stress, which can contribute to short tear break-up time. However, other aspects of sedentary behaviour may contribute to the risk of dry-eye, therefore physical inactivity may not be causal. For example, increased visual display unit use can reduce blinking rate and consequently dryness of the ocular surface increases. Sano et al. (2018) recruited office workers suffering from dry-eye symptoms to a 10-week home based core exercise intervention, and found an improvement in subjective dry-eye symptoms. Similarly, Kawashima et al. (2018) enlisted office workers for a two-month multi-disciplinary lifestyle intervention including

increased physical activity and also found improvements in subjective symptoms. These intervention studies add to the observational data reported by Kawashima et al. (2014), but objective measurements are required to determine the efficacy of exercise in attenuating dry-eye symptoms. It remains unknown whether an acute bout of exercise influences symptoms of dry-eye. Theoretically, exercise induced increases in core body temperature may increase ocular surface temperature (OST), improving the fluidity of the meibomian lipids within the tear film and inhibit evaporation from the eye (A. Bron, Tiffany, Gouveia, Yokoi, & Voon, 2004). Conversely the risk of dry-eye could be increased by exercise because increased OST may elevate the rate of tear evaporation (Borchman et al., 2009).

The aim of this study was to investigate the effect of acute exercise on environmentally induced symptoms of dry-eye.

Methods

Participants

Twelve (seven male, five female; mean ± SD age 26.7 ± 5.7 years, mass 72.9 ± 11.6 kg, stature 1.73 ± 0.09 m, peak power output 294.0 ± 54.8 W) non dry-eye sufferers volunteered to take part in this study. All participants were screened using the ocular surface disease index (OSDI) survey to confirm that they did not suffer from dry-eye. Participants undertook a preliminary Schirmer test to familiarise them to the procedure and ensure that they produced a reading of at least 5 mm to allow for later analysis (see procedures for full description). All participants provided written and verbal consent, and all procedures were approved by the ethics committee at Northumbria University (reference 897).

Experimental Design

Participants reported to the laboratory on four occasions; (i) preliminary measures and maximal aerobic exercise test, (ii) control condition (CONT; seated for 60 min at 25°C 40% RH), (iii) dry condition (DRY; seated for 60 min at 25°C 20% RH), (iv) exercise condition (EXER; seated for 40 min, 100 W warm up for 5 min, and cycle at 40% Δ (190.4 ± 35.7 W) for 15 min under dry conditions). Visits 2-4 were completed in a counterbalanced randomised order (https://www.randomizer.org/) and took place within an environmental chamber (Peak

Performance Chamber Series 2009, T.I.S. Services, UK) to control the temperature and RH. The environmental conditions were modified from (Abusharha & Pearce, 2013) to environmental conditions (temperature and RH) that could be maintained within the environmental chamber during the exercise trial. The following measurements were taken at the end of each 60 min exposure; (i) tear production, (ii) core temperature, (iii) OST, (iv) perceived dryness of the eye.

Procedures

Prior to prescription of exercise, participants completed an incremental exercise test on a cycle ergometer (Velotron Pro cycle ergometer, Racermate Inc., USA) to volitional exhaustion. Participants performed a warm up of unloaded cycling (2-5 minutes) and progressed 30 $W.min^{-1}$ in a ramped fashion until volitional exhaustion or until 50 RPM could not be maintained by the participant. Expired gas was collected and analysed using an online breath-by-breath gas analyser (Cortex Metalysyer 3B, Biophysik, Germany). Breath-by-breath data were averaged using a rolling average of the middle 5 of every 7 breaths. The ventilatory anaerobic threshold was identified using the modified V-slope method (Beaver, Wasserman, & Whipp, 1986) and confirmed with ventilatory equivalents for the rate of oxygen uptake ($\dot{V}_E/\dot{V}O_2$) and carbon dioxide production ($\dot{V}_E/\dot{V}CO_2$) (Whipp, Ward, & Wasserman, 1986). Maximum $\dot{V}O_2$ ($\dot{V}O_2max$) was identified as the highest oxygen consumption achieved during exercise, averaged over 30 s (Midgley, McNaughton, & Carroll, 2007). The 40% Δ workload was calculated by identifying the work rate at the ventilatory anaerobic threshold (140.1 ± 26.7 W), subtracting 2/3 of the ramp rate to account for the delay in oxygen kinetics (Whipp, Davis, Torres, & Wasserman, 1981) and calculating 40% of the difference between this value and the peak work rate achieved. This was to ensure that participants exercised at a similar relative intensity that could be sustained for 15 minutes and was within the heavy exercise intensity domain (Burnley & Jones, 2007).

Tear production was quantified using 40 mm Schirmer strips (Praxisdienst, UK) which were placed 5 mm inside the lower eye lid. The eye was kept closed for 5 min as per standard procedures (Serin, Karsloglu, Kyan, & Alagöz, 2007). Once the reading had been taken, each strip was placed inside a 0.5 ml Eppendorf tube with a hole puncture in the bottom. This was then placed in a 1.5 ml Eppendorf tube and centrifuged at 13,000 rpm for 5-min (Posa et al., 2013). The resultant supernatant was immediately stored at -80 °C for later analysis of MMP-

9 using a commercially available ELISA kit (R & D Systems, USA). MMP-9 was only detectable in all trials for eight participants hence n = 8 for this variable.

Core temperature was measured using a tympanic thermometer (Braun Thermoscan 5, Germany), and OST from the centre of the eye using a thermal imaging camera (TG165 Imaging IR thermometer, FLIR Systems, USA) eight seconds after a blink (Purslow, Wolffsohn, & Santodomingo-Rubido, 2005). Perceived dryness of the eye was determined using the Symptom assessment in dry-eye (SANDE) questionnaire (Amparo, Schaumberg, & Dana, 2015), where participants answered two questions on a 100 mm visual analogue scale; (i) indicate how often in the last hour your eyes feel dry and/or irritated, and (ii) indicate how severe you feel your symptoms of dryness and/or irritation are. Change in body mass during the EXER trial was taken to ascertain if dehydration contributed to any of the findings.

Data Analysis

An *a priori* sample size estimation identified that 12 participants would be suitable to identify a change of 5 mm in the Schirmer test with 80% power. This was chosen because the upper range of the variation of resting Schirmer measures across three laboratory visits in 14 healthy participants was 3 mm (Serin et al., 2007). A similar difference has been reported across stages of the menstrual cycle (Versura, Fresina, & Campos, 2007). Data were checked for normal distribution via visual inspection of box plots and Q-Q plots. Data were analysed using PASW Statistics 22.0 for Windows (SPSS, Inc., Chicago, IL, USA). None of the variables were normally distributed so differences were determined using the Friedman test, and where a significant ($P < 0.05$) X^2 was found post-hoc Wilcoxon signed ranks tests were applied with a Bonferroni correction ($P < 0.017$). Data are presented as the median and interquartile (IQ) range.

Results

The median and IQ range for each variable are presented in Table 1. Tear volume measured using the Schirmer strips was not significantly different between conditions ($X^2 = 2.591$, $P = 0.274$). Similarly, core temperature ($X^2 = 3.511$, $P = 0.173$) and OST ($X^2 = 0.304$, $P = 0.859$) were comparable between conditions. There was a significant main effect between conditions for the frequency ($X^2 = 11.73$, $P = 0.003$) and severity ($X^2 = 9.8$, $P = 0.007$) of perceived dry-

eye symptoms. The post-hoc threshold was not met by the severity data ($P \geq 0.028$), but the frequency of symptoms was higher in the DRY compared to CONT and EXER conditions ($P \leq 0.008$). The concentration of MMP-9 was different between conditions ($X^2 = 7.75$, $P = 0.021$), specifically it was higher in DRY compared to CONT ($P = 0.012$). Percentage dehydration as indicated by change in body mass during EXER was negligible (0.27% on average). The heart rate in the final minute of the exercise trial was 173 ± 13 beats per minute.

Discussion

The main findings of this study are that exposure to a desiccating environment of 20% RH did not influence tear volume measured by Schirmer strips. However, 20% RH appeared to increase the perception of the frequency of dry-eye symptoms and presence of MMP-9 in tears at rest. Interestingly, the addition of exercise in the final 20 minutes exposure may attenuate these symptoms.

Tear volume, as measured by Schirmer strips, was not different between trials. This is in contrast to previous literature, where tear production was reduced in resting dry conditions using similar methods (Abusharha & Pearce, 2013). The disparity may be due to the modest RH (20%) used in this experiment, compared to the RH (5%) employed by Abusharha and Pearce (2013). Despite no differences in tear volume, the DRY condition elicited a greater perception of dry-eye symptom frequency, and increased MMP-9 in tears. Therefore, an arguably more ecologically valid RH used in the present study appears to initiate symptoms of dry-eye.

The perception of dry-eye symptom frequency was reduced in the EXER trial compared to DRY. This supports previous research that found increasing physical activity can reduce the perception of symptoms (Kawashima et al., 2018; Sano et al., 2018). However, there is risk of a placebo effect on perceptual responses with exercise. To account for this, we included MMP-9 as an objective biological marker of dry-eye. MMP-9 concentrations were higher in DRY compared to CONT. Although MMP-9 was not different between EXER and DRY, the EXER condition was also not different to CONT. Therefore, it could be viewed that exercise may play a protective role in dry environments. The mechanism for the potential protective role of exercise against dry eye is unclear. Our proposed theory of increased core temperature increasing OST remains untested as core temperature was similar between conditions.

Hypothetical mechanisms could include changes in blinking rate, pro- and anti-inflammatory cytokine release during exercise, or changes in intraocular pressure and blood flow. However, this is speculation as these variables were not measured. Furthermore, tympanic thermometers have been shown to vary from rectal temperature by up to 0.67°C (Ganio et al., 2009), so the originally proposed mechanism cannot be discounted.

This is the first pilot study of its kind and it is important to note the limitations. The sample size estimation was based on Schirmer test results. Therefore, our secondary findings pertaining to MMP-9 are at risk of type 1 error. There was no difference in MMP-9 between DRY and EXER therefore the potential benefits of exercise on reducing dry-eye should be interpreted with caution. Similarly, the small sample size of this pilot study and because MMP-9 was a secondary outcome measure, type 2 error cannot be discounted. Another factor limiting the interpretation of the MMP-9 results is the absence of time course data following environmental condition with or without exercise exposure. During practice trials the conditions were set at 21°C and 10% RH, however these conditions were difficult to maintain when participants were exercising. Increasing the RH to 20% was not strictly an issue as it made the study more ecologically valid than past research, but the increase in temperature to 25°C perhaps reduces ecological validity for some parts of the world. For this pilot trial, it was important to ensure that temperature could be matched between conditions.

We believe that these findings are interesting and merit further investigation. For MMP-9 to be used a primary outcome measure in future studies, it would be useful to have reproducibility data on this important biological marker. Additionally, further research is warranted to extend the data collection period beyond the time point immediately following the one hour condition exposure to allow further insight into the time course of any beneficial effects. It would also assist in determining whether the changes in perceived symptoms continue after the distraction of taking part in exercise.

In conclusion this study provides some evidence exercise may attenuate acute perceptions of dry-eye symptoms and MMP-9 in tears when exposed to a desiccating environment. Limitations to this pilot work have been acknowledged and a larger scale study is warranted before any firm conclusions are drawn. However perhaps as important is that the results provide no evidence that exercise exacerbates acute symptoms of dry-eye, although it is acknowledged that the exercise stimulus was relatively low.

Competing Interests

None declared. No external funding was received for this experiment.

Author Contributions

DP, IW, AOD and AM designed the experiment. DP, IW, AOD, ES, EJ and TH collected the data. DP drafted the manuscript and all other authors contributed to revisions. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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