Does air gas aesthesiometry generate a true mechanical stimulus for corneal sensitivity measurement?

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Air gas aesthesiometry was designed to overcome some of the limitations of the 'gold standard', the Cochet-Bonnet aesthesiometer, such as the risk of abrasion of the epithelial surface, alignment and precision difficulties, a limited stimulus range and the influence of ambient humidity on how the nylon filament bends.[1,2]

The Belmonte Ocular Pain Meter aesthesiometer (OPM; Deriva Global S.L., Valencia, Spain) was the first commercially available non-contact instrument, however it is no longer available. It uses a pulse of pressurised air directed through an air-jet located close to the eye to stimulate the ocular surface using a cooling stimulus. The latter can be heated to give a mechanical or warming stimulus, or mixed with CO_2 to provide a chemical stimulus. Depending on which type of nerve endings, in the cornea or ocular surface, are to be excited, different stimulus temperatures can be chosen. A cooling stimulus with a temperature lower than ocular surface temperature (OST) will excite the temperature sensitive C fibres, whereas an air gas stimulus equal to OST should be sensed by the mechanically sensitive Aδ fibres when a sufficient degree of corneal deformation can be produced by the air gas stimulus. For true mechanical corneal sensitivity measurement, the airflow stimulus temperature of the aesthesiometer must equal corneal temperature at the corneal surface to avoid additional response from temperature-sensitive nerves.[3] However, the exact temperature that the air stimulus of the OPM should have to deliver an isolated mechanical stimulus, is not clear and indeed, if a truly mechanical stimulus can be generated with an air gas aesthesiometer is unknown.

In their review article, Purslow and Wolffsohn[4] stated that OST is influenced by the following external and internal factors: heat transfer from adjacent structures, blinking (warm tears being spread across the ocular surface during each blink) and after each blink, temperature of the ambient environment (0.15 to 0.20°C change in OST per 1°C

change in room temperature), changing blood flow to the eye or head, and diurnal

variation (OST rises during the course of the day).[4] Obviously, an air gas stimulus will

lead to a corneal cooling response if its temperature is set at room temperature, since

this temperature will be lower than that of the corneal surface (typically 22°C room

temperature vs 34°C OST). If, however, the stimulus temperature is chosen to match

corneal temperature, it is proposed that there will be no cooling effect and that the

resulting stimulus will only cause mechanical deformation and stimulate the Aδ fibres,

but not the cold-sensitive C fibres. However, it is not yet clear if this is what happens

with the mechanical stimulus heated to match OST, or if it can still cause a cooling

response resulting from tear film thinning. Furthermore, it is possible that stimulus

temperature may vary upon arrival on the ocular surface, depending on the air flow rate,

the distance to the ocular surface or the duration of presentation.

There are some discrepancies between different research groups as to what temperature

the air stimulus should be in order to deliver a mechanical stimulus. Feng and Simpson,

as well as Stapleton et al., recommended a stimulus temperature of 50°C,[5,6] and in 1999,

Belmonte et al. also used 50°C.[2] However, the manufacturers of the OPM recommend

43°C, which equates to a temperature increase of 19°C above a room temperature of

24°C (OPM User Manual, Deriva Global S.A.).

The aim of this study was to determine whether the Belmonte OPM delivers true

mechanical stimuli, which are independent of airflow rate for a specific stimulus

duration:

To this purpose, the following objectives were performed:

1) to determine the stimulus temperature that would induce the least change in OST or

equal to OST with constant stimulus duration, distance to the ocular surface and airflow

rate. This stimulus temperature would be most likely to be suitable for the measurement of mechanical corneal sensitivity.

2) to evaluate if OST remains unchanged with one defined stimulus temperature with different stimulus durations, employing varying airflow rates. This would give some indication if a corneal sensitivity threshold may be determined with means of the air stimulus generated by the OPM whilst avoiding a change in OST.

METHODS

This was a prospective clinical cohort study. The clinical experiments took place in two parts: a preliminary part A and B. For the preliminary part A, six volunteers were recruited and the effect of air gas stimuli of different temperatures on ocular surface temperature (OST) was investigated at a set level of stimulus duration (3s), a set distance to the ocular surface (4mm) (measured by a scale held to the side of the instrument and subject's eye) and a set airflow rate (60ml/min). For part B, an additional ten subjects were recruited and the effects of stimulus duration (3 and 5s) and airflow rate (30, 60, 80 and 100ml/min) on OST were assessed at a set distance of 4mm, by applying the stimulus temperature most likely to match OST (as obtained from study part A) on all 16 subjects.

Ethical approval was obtained from the human research ethics committee of the School of Optometry and Vision Sciences, Cardiff University for both study parts A and B (project number 1368). The participants were volunteers from the student pool of the School of Optometry, University of Applied Sciences Northwest Switzerland in Olten, who were invited for participation via email. All subjects invited to take part in the study filled in the Ocular Surface and Disease Index (OSDI)[7] questionnaire and were

given a participant information sheet explaining the study prior to providing signed

consent.

The age range was limited to between 20 and 39 years, since OST[8] and tear film

stability[9] have been found to decrease with age. Exclusion criteria for participation in

this study were: history of previous ocular surgery including refractive surgery, eyelid

tattooing, eyelid surgery or corneal surgery; previous ocular trauma; Sjögren's

Syndrome (absence of dry mouth), rheumatoid arthritis, diabetes or ocular infections;

current or previous condition known to affect the ocular surface and/or tear film; a score

≥ 15.0 on the Ocular Surface Disease Index (OSDI) questionnaire;[7] medication or use of

eye drops known to affect the ocular surface and/or tear film; pregnancy (on self-

report); contact lens (CL) wear one day prior or on the day of this study, as this may

affect tear film stability. This short cessation of CL wear was deemed sufficient, as this

study did not evaluate ocular surface sensitivity.

Humidity levels and room temperature were controlled to maintain normal office

environmental limits (by means of air conditioning), as these variables have been shown

to influence OST,[10-12] showing a typical increase of 0.15 to 0.2°C per 1°C increase in

room temperature.[4] The level of humidity during this study was 38.60±2.5%. Ambient

temperature was recorded before the measurement of each stimulus type on each

individual subject. It was found to be 23.06±0.34°C during part A, and 23.11±0.32°C

during part B. The OPM used for the following experiments has been equipped with the

addition of a temperature sensor, fitted on the outside of the instrument housing, for

measurement of ambient room temperature.

5

Real-time measurements of OST were carried out on all subjects, using a self-

calibrating thermal infrared camera (FLIR A310, FLIR Systems, Wilsonville, USA;

thermal resolution 0.08°C, temporal resolution 30Hz; spatial resolution 320x240 pixel,

corneal emissivity 0.95). The camera could not be positioned perpendicular to the

cornea, but had to be offset a little to the side and therefore measured OST from an

oblique position. This was necessary, since the nozzle of the aesthesiometer must be

positioned directly in front of the cornea, perpendicular to the ocular surface, which

explains the oblique position of the thermal camera, and its long working distance of

20cm to the corneal surface. OST was only measured on the right eye and the

participating subjects were asked to close their eyes immediately after each

measurement for 20s in order for the tear film to recover after each stimulus

presentation. In order to ensure a complete and stable tear film over the cornea, the

subject was asked to make a full, but unforced blink, following which (within 1-2s) the

stimulus was presented.[13] The correct positioning of the direction of gaze was

controlled with aid of a correctly positioned fixation target on the wall.

Air gas stimulus temperature

Stimulus temperature can be regulated by the choice of 'delta'. 'Delta' represents the

number of degrees (°C) by which the OPM will heat the air gas stimulus above the

ambient temperature indicated by the external thermometer attached to the instrument.

The effect of different deltas on OST was explored during study part A, by applying

delta 5, 10, 15, 20 and 30. Since it was expected that the air stimulus would cool down

to a certain degree between leaving the nozzle of the aesthesiometer and arriving at the

corneal surface, and the amount of this temperature change was not known, the exact air

stimulus temperature could not be calculated by simply adding room temperature and

the delta of the stimulus temperature.

6

Analysis of OST during stimulus presentation

The thermal images were analysed using a purpose-designed computer programme

(ThermaCAM Researcher Pro Version 2.9, FLIR Systems, 2006), which displayed

approximately six images per second. The corneal area targeted by the air stimulus

(Figures 1 and 2) was marked and analysed during stimulus presentation. A legend next

to the image indicated the precise positive or negative temperature changes in °C within

this area.

OST change could be analysed with the aid of the manufacturer's analysis software,

which gave a time plot for the OST change for the full duration of the temperature

recording during the entire length of stimulus presentation. By browsing through the

recording, the area of OST change could be identified and subsequently marked. The

size of this marked area was chosen to catch the localised temperature change induced

by the air stimulus. The scale for the temperature range could then be locally adjusted

within this area ('local auto adjustment'), which allowed a more precise visual

identification of the area where the temperature change took place (Figures 1 and 2).

The minimum and maximum OSTs (°C) within this marked area were displayed for

each image during a stimulus presentation. Before each stimulus presentation, the

baseline minimum and maximum OST were displayed for each measurement within the

area of interest. For the analysis of OST change during a cooling stimulus, the baseline

temperature value of the minimum temperature within the area was noted before

stimulus presentation. During a cooling stimulus presentation, the maximum OST

change was noted by recording the lowest temperature value for the minimum

temperature within the area. The difference between the baseline and the lowest

temperature represented the maximum temperature decrease. Correspondingly, during a

stimulus warmer than the ocular surface, the maximum temperature change was

determined by noting the difference between the lowest (baseline) and highest value of

the maximum temperature within the area during stimulus presentation. These limits

ensured that the maximum effect of any OST change occurring during stimulus

presentation was detected.

Preliminary study part A

OST was measured during stimulus presentation with increasing levels of stimulus

temperature, i.e. rising levels of the following deltas: 5, 10, 15, 20 and 30°C. Stimulus

distance to the ocular surface was set at 4mm, stimulus duration was set at 3s and

airflow rate was set at 60ml/min. Each measurement was carried out three times.

Study part B

The two deltas that were found to provoke least OST change during part A were then

applied with two different stimulus durations of 3 and 5s and with the airflow rates of

30, 60, 80 and 100ml/min in 16 subjects. These airflow rates represent a range that

includes normal levels of corneal sensitivity thresholds.[14] A stimulus duration of 3s was

recommended by the manufacturer and was used by Gallar et al.[15]; the application of the

longer stimulus duration of 5s has not been published before and was chosen

experimentally in this study. As in part A, each measurement was carried out three

times. The order of measurements was randomised for different stimulus characteristics

(duration, and temperature), however the order of airflow rates was kept constant, in

rising order, just like the air stimuli would be presented during corneal sensitivity

threshold measurements: 30, 60, 80 and, lastly, 100ml/min.

Statistical analysis

The data were tested for normal distribution (Shapiro-Wilk Test). A general linear

model (two-way ANOVA) was applied for the normally distributed data and

subsequently post-hoc t-tests with Bonferroni corrections were carried out (SPSS

Version 20, Chicago, IL, USA).

RESULTS

Preliminary study part A

Six subjects participated in this study, and the average subject age was 27.10±3.81

years, three subjects were female. Mean OST at baseline was found to be within a

normal range (Table 1) that is believed to be 32.9-36°C.[4] When delta was set at 5 and

10, OST decreased. At delta 15, it increased very slightly and more considerably at delta

20 and 30 (Table 1, Figure 3). A near linear increase in corneal temperature was

observed with increasing levels of delta.

Table 1: Mean OST at baseline and mean OST / OST change during stimulus presentation with increasing levels of delta.

Mean OST at baseline and OST change during stimulus presentation with increasing levels of delta			
Delta	**Mean OST at baseline ± standard deviation (°C)**	**Mean OST with stimulus ± standard deviation (°C)**	**Mean OST change ± standard deviation (°C)**
5	**35.23±0.68**	**34.26±0.49**	**-0.97±0.30**
10	**35.30±0.80**	**35.10±0.70**	**-0.20±0.13**
15	**36.24±0.45**	**36.32±0.42**	**+0.08±0.05**
20	**36.52±0.57**	**37.28±0.58**	**+0.77±0.43**
30	**36.98±0.53**	**39.09±1.17**	**+2.11±0.82**

Study part B

During the preliminary study part A, delta 10 and delta 15 were identified to cause the least OST change and they were subsequently applied. Sixteen subjects participated in this study, however two of them experienced tearing during stimulus presentation. The data were analysed for outliers using boxplots (SPSS, Version 20) and the two subjects, identified as outliers, were consequently removed from the analysis. The mean age of the remaining 14 subjects was 25.14±2.18 years, seven were female.

OST changes with delta 10

The changes in localised absolute OST for the overall group were found to be statistically significant for all stimulus characteristics (p<0.001, F=50.50 for 3s duration; p=0.021, F=8.70 for 5s duration; two-way ANOVA).

Although the absolute OST changes during stimulus presentation were small, they were all statistically significant (post-hoc paired t-test; Tables 2 and 3), with the exception of the stimulus setting at 5s duration with airflow 30ml/min (Table 3). The localised OST cooling was more marked with the longer stimulus duration of 5s, and was also more pronounced with the higher airflow rate (Table 3 and Figure 4).

Table 2: Mean OST changes for stimulus duration of 3s (delta 10).

Mean OST at baseline and OST change with varying levels of airflow rates for 3s duration (delta 10)				
Airflow rate (ml/min)	OST at baseline ± standard deviation (°C)	OST with stimulus ± standard deviation (°C)	OST change ± standard deviation (°C)	p-value
30	34.80±0.85	34.60±0.86	-0.23±0.12	<0.001
60	34.61±0.81	34.22±0.87	-0.36±0.14	<0.001
80	34.73±0.91	34.17±1.03	-0.49±0.28	<0.001
100	34.67±0.90	33.96±1.14	-0.62±0.40	<0.001

Table 3: Mean OST changes for stimulus duration of 5s (delta 10).

Mean OST at baseline and OST change with varying levels of airflow rates for 5s duration (delta 10)				
Airflow rate (ml/min)	OST at baseline ± standard deviation (°C)	OST with stimulus ± standard deviation (°C)	OST change ± standard deviation (°C)	p-value
30	34.50±0.48	34.15±0.51	-0.38±0.42	0.456
60	34.57±0.54	34.07±0.42	-0.60±0.43	0.027
80	34.57±0.55	34.07±0.51	-0.64±0.26	0.006
100	34.63±0.64	33.98±0.63	-0.80±0.51	0.004

OST changes with delta 15

The changes in localised absolute OST for the overall group were found to be

statistically significant for all stimulus characteristics (p<0.001, F=97.95 for 3s

duration; p<0.001, F=63.72 for 5s duration; two-way ANOVA).

Although the absolute OST changes during stimulus presentation were also small, they
were all statistically significant (post-hoc paired t-test; Tables 4 and 5), with the
exception of the stimulus setting at 3s duration with the airflow rate of 30ml/min (Table
4). The localised increase in OST was similar with the longer stimulus duration of 5s
(Tables 5; Figure 5). OST change was also more pronounced, as the airflow rate
increased (Figure 5).

Table 4: Mean OST changes for stimulus duration of 3s (delta 15).

Mean OST change with varying levels of airflow rates for 3s duration (delta =15)				
Airflow rate (ml/min)	OST at baseline ± standard deviation (°C)	OST with stimulus ± standard deviation (°C)	OST change ± standard deviation (°C)	p-value
30	35.24±0.79	35.24±0.78	0.00±0.07	1.000
60	35.09±0.75	35.27±0.75	0.18±0.17	0.002
80	35.16±0.82	35.47±0.83	0.32±0.13	<0.001
100	35.17±0.87	35.59±0.92	0.43±0.10	<0.001

Table 5: Mean OST changes for stimulus duration of 5s (delta 15).

Mean OST change with varying levels of airflow rates for 5s duration (delta =15)				
Airflow rate (ml/min)	OST at baseline ± standard deviation (°C)	OST with stimulus ± standard deviation (°C)	OST change ± standard deviation (°C)	p-value
30	34.74±0.56	34.81±0.56	0.07±0.06	0.012
60	34.83±0.53	34.97±0.54	0.14±0.09	0.003
80	34.91±0.49	35.19±0.47	0.28±0.11	<0.001
100	34.90±0.55	35.34±0.54	0.44±0.10	<0.001

When comparing localised OST change induced by the air gas stimulus (Figure 6), it was noted that it was more pronounced with delta 10 than it was with delta 15. In addition, more variability in OST change between subjects was observed with delta 10, displayed by the higher standard deviations.

A two-tailed post-hoc power calculation was carried out for the standard stimulus characteristics, for each of the airflow rates applied, and a statistically significant difference was obtained (α=0.05; n=14; G*Power 3.1):

For airflow rate of 60ml/min:

- OST difference of 0.18±0.17°C; effect size = 1.059; power = 0.95

For airflow rate of 80ml/min:

- OST difference of 0.32±0.13°C; effect size = 2.46; power = 1.0

For airflow rate of 100ml/min:

- OST difference of 0.43±0.10°C; effect size = 4.3; power = 1.0

DISCUSSION

This clinical study was aimed at measuring stimulus temperature of the air gas stimulus generated by the Belmonte OPM aesthesiometer by recording changes in OST during stimulus presentation with the use of an infrared thermal camera. Air stimuli cooler and warmer than the ocular surface were generated from a 4mm distance to the cornea, with two different durations (3 and 5s) and different airflow rates (30, 60, 80 and 100ml/min). The resulting changes in OST were analysed statistically. Additionally, it was of particular interest to establish if it was possible to generate air stimuli matching OST.

During the preliminary study part A, it could be observed that stimulus temperature set at delta 15 created the least OST change, and while the air stimuli with delta 10 created a larger OST change than with delta 15, the effect was still small enough to qualify for further investigation. Hence, study part B was conducted in order to test the effect of the other stimulus characteristics such as stimulus duration and airflow rate with delta set at 10 as well as 15, in order to evaluate if a stimulus could be generated that would equal OST and would therefore be suitable to create a purely mechanical stimulus during corneal sensitivity threshold measurement.

elta 15 could be confirmed as the temperature setting inducing the smallest, but still statistically significant, OST change with all stimulus characteristics. Increasing the stimulus duration of the delta 15 stimulus to 5s did not produce a further reduction in OST across the airflow rates tested in this study. Crucially, this means that there is not an exclusively mechanical stimulation with this type of air stimulus, but there must be a contribution from the temperature sensitive corneal C-fibres reacting to the air-stimulus. As a consequence, OST change cannot be eliminated, even if the temperature effect of an air stimulus with delta 15 and a room temperature of 23-24°C is small, with a maximum mean difference of 0.44°C. Moreover, this difference has to be considered, since the smallest temperature change likely to excite the temperature sensitive nerve endings has been shown to be 0.1°C in cats[16,17], and the smallest OST difference to be sensed by the ocular surface in humans as 0.3°C.[18] This applies especially, if air stimuli with air flow rates of ≥ 80ml/min are employed.

Clearly, the recommended manufacturer's setting of delta 19 was not found to be ideal for mechanical threshold testing, since, in this study, the localised mean OST change with delta 20 was +0.77±0.43°C.

A more systematic problem with this form of aesthesiometry is that the OST change

increased proportionally to the increase in airflow rate applied. Stimulus intensity is

controlled by airflow rate, but the results show that airflow itself is also the prime factor

in changes in OST change produced by the stimulus. This means that during corneal

sensitivity threshold measurement, with application of a double staircase method,[19]

there will be a variable influence of OST change affecting the mechanical corneal

sensitivity threshold obtained from the measurement. So even if the stimulus is heated

by a fixed delta, that delta would only be suitable for one airflow rate. To truly remove

any temperature difference between the air gas stimulus and the ocular surface, delta

would need to adjust in synchronisation with the airflow. Stimulus temperature would

have to be adjusted proportionally with increasing airflow rates. Moreover, in view of

the physiological variability observed between subjects, some initial calibration step

would be required that would be able to assess OST as the baseline for the stimulus,

rather than the ambient room temperature. However, even if a stimulus with a

temperature equal to the ocular surface can be generated, it would be necessary to show

that this air gas stimulus did not generate an additional response from the temperature

sensitive nerve endings caused by thinning of the tear film.

Although the findings of this study only apply to the Belmonte OPM aesthesiometer,

they could have an impact on how ocular surface sensitivity thresholds obtained with

other air gas aesthesiometers should be interpreted, when the aim was or is going to be

to apply an exclusively mechanical stimulus.

CONCLUSIONS

This study shows evidence that a true mechanical threshold for corneal sensitivity

cannot be established with the air stimulus of the Belmonte OPM aesthesiometer and

that the air gas stimulus is likely to have a non-mechanical, thermal component. The

manufacturer's temperature setting for a mechanical stimulus would have to be

amended to a system that allows synchronisation between stimulus temperature and

airflow rate, and it would have to be ensured that the air gas stimulus does not excite the

temperature sensitive nerve endings as a result of tear film thinning.

The best delta 15 setting established in this study is also considerably different from the

OPM recommended setting of delta 19. In this study, the mean OST change was

+0.77±0.43°C with delta 20 at this approximate delta setting.

References

1. Murphy PJ, Patel S, Marshall J. A new non-contact corneal aesthesiometer (NCCA). Ophthalmic Physiol Opt. 1996;16(2):101–107.

2. Belmonte C, Acosta MC, Schmelz M, Gallar J. Measurement of corneal sensitivity to mechanical and chemical stimulation with a CO_2 esthesiometer. Invest Ophthalmol Vis Sci. 1999;40(2):513–519.

3. Murphy PJ, Lawrenson JG, Patel S, Marshall J. Reliability of the non-contact corneal aesthesiometer and its comparison with the Cochet-Bonnet aesthesiometer. Ophthalmic and Physiological Optics. 1998;18(6):532–539.

4. Purslow C, Wolffsohn JS. Ocular surface temperature. Eye & Contact Lens: Science & Clinical Practice. 2005;31(3):117–123.

5. Feng Y, Simpson TL. Nociceptive sensation and sensitivity evoked from human cornea and conjunctiva stimulated by CO_2. Invest Ophthalmol Vis Sci. 2003;44(2):529–532.

6. Stapleton F, Tan ME, Papas EB, et al. Corneal and conjunctival sensitivity to air stimuli. British Journal of Ophthalmology. 2004;88(12):1547–1551.

7. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol. 2000;118(5):615–621.

8. Aliò J, Padron M. Influence of age on the temperature of the anterior segment of the eye. Measurements by infrared thermometry. Ophthalmic Res. 1982;14(3):153–159.

9.   Patel S, Farrell JC. Age-related changes in precorneal tear film stability. Optom

       Vis Sci. 1989;66(3):175–178.

10.   Holly FJ. Tear film physiology. American Journal of Optometry and

       Physiological optics. 1980;57(4):252–257.

11.   Doane MG. Blinking and the mechanics of the lacrimal drainage system.

       Ophthalmology. 1981;88(8):844–851.

12.   Schwartz B. Environmental temperature and the ocular temperature gradient.

       Arch Ophthalmol. 1965;74:237–243.

13.   Murphy PJ, Ntola AM. Prolonged corneal anaesthesia by proxymetacaine

       hydrochloride detected by a thermal cooling stimulus. Cont Lens Anterior Eye.

       2009;32(2):84–87.

14.   Tesón M, Calonge M, Fernandez I, Stern ME, González-García MJ.

       Characterization by Belmonte's gas esthesiometer of mechanical, chemical, and

       thermal corneal sensitivity thresholds in a normal population. Invest Ophthalmol

       Vis Sci. 2012;53(6):3154–3160.

15.   Gallar J, Tervo T, Neira W, Holopainen JM, Lamberg ME, Minana F, Acosta

       MC, Belmonte C. Selective changes in human corneal sensation associated with

       herpes simplex keratitis. Invest Ophthalmol Vis Sci. 2010;51(9):4516-4522.

16.   Gallar J, Pozo MA, Tuckett RP, Belmonte C. Response of sensory units with

       unmyelinated fibres to mechanical, thermal and chemical stimulation of the cat's

       cornea. J Physiol (Lond). 1993;468:609–622.

17.   Belmonte C, Acosta MC, Gallar J. Neural basis of sensation in intact and injured

corneas. Exp Eye Res. 2004;78(3):513–525.

18. Murphy PJ, Patel S, Morgan PB, Marshall J. The minimum stimulus energy required to produce a cooling sensation in the human cornea. Ophthalmic Physiol Opt. 2001;21(5):407–410.

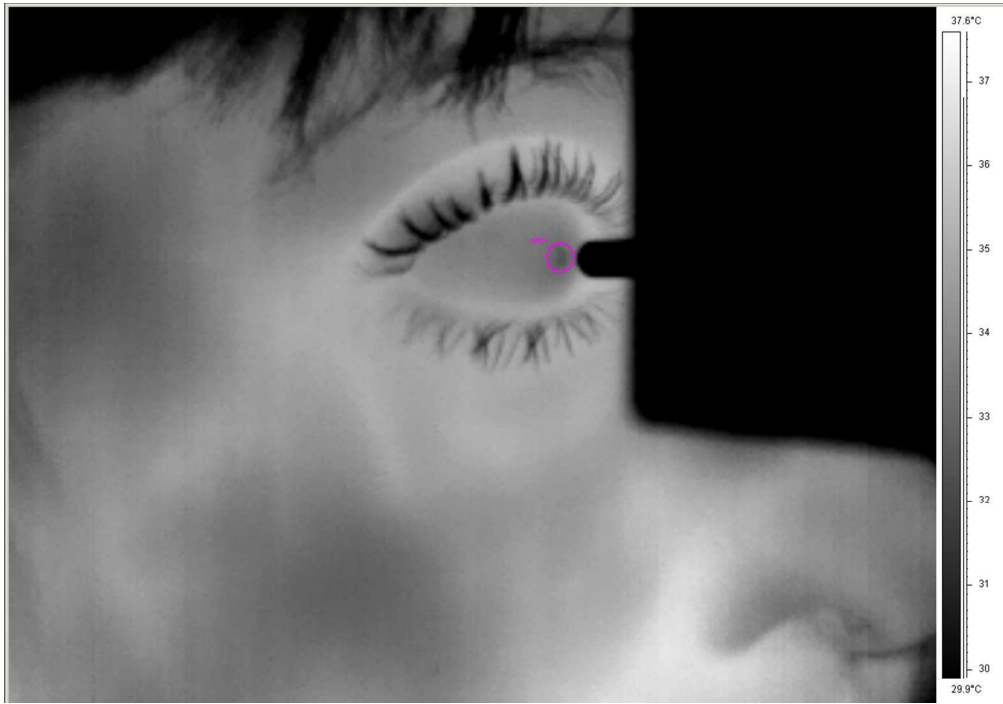19. Murphy PJ. An Examination of Human Corneal Sensitivity by Non-Invasive Methods. PhD Thesis, Glasgow University; 1996:1–245.

Figure 1: Example of localised OST decrease during a cooling air stimulus before local auto adjustment of the area of interest.

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434x303mm (72 x 72 DPI)

Figure 2: Example of localised OST decrease during a cooling air stimulus after local auto adjustment of the area of interest.
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430x303mm (72 x 72 DPI)
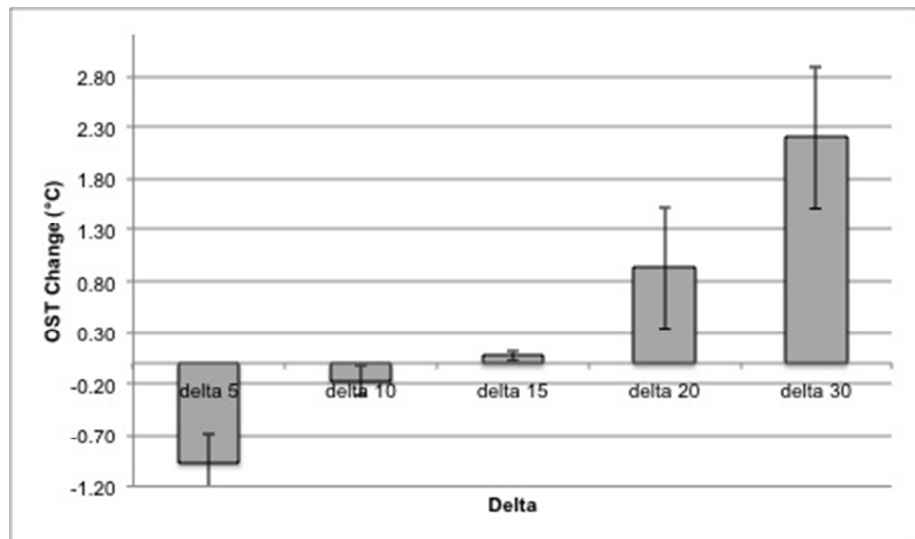
Figure 3: Localised OST change with increasing levels of delta.
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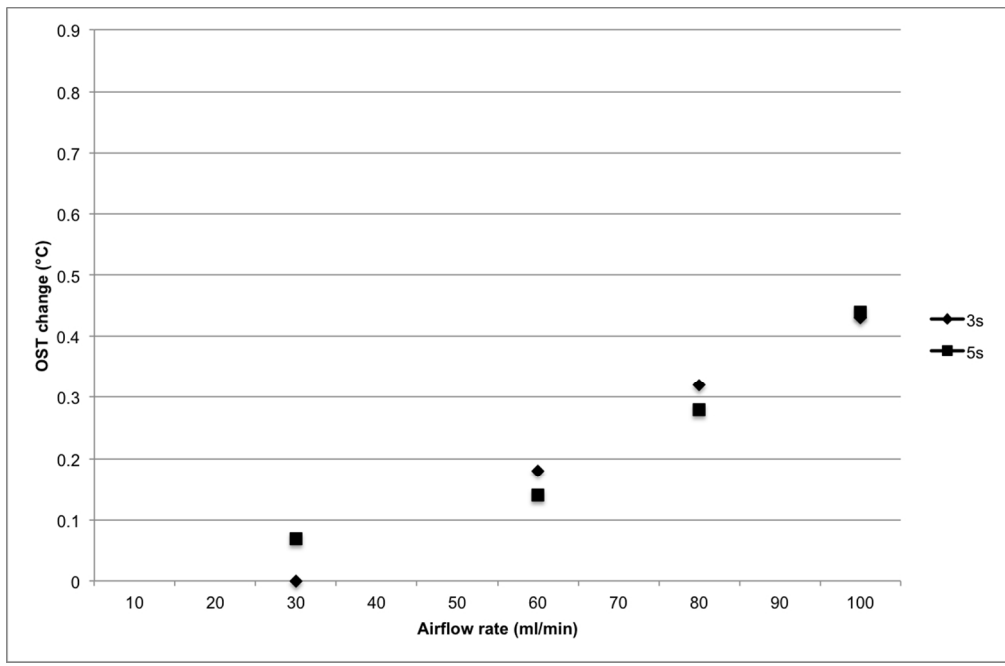160x94mm (72 x 72 DPI)
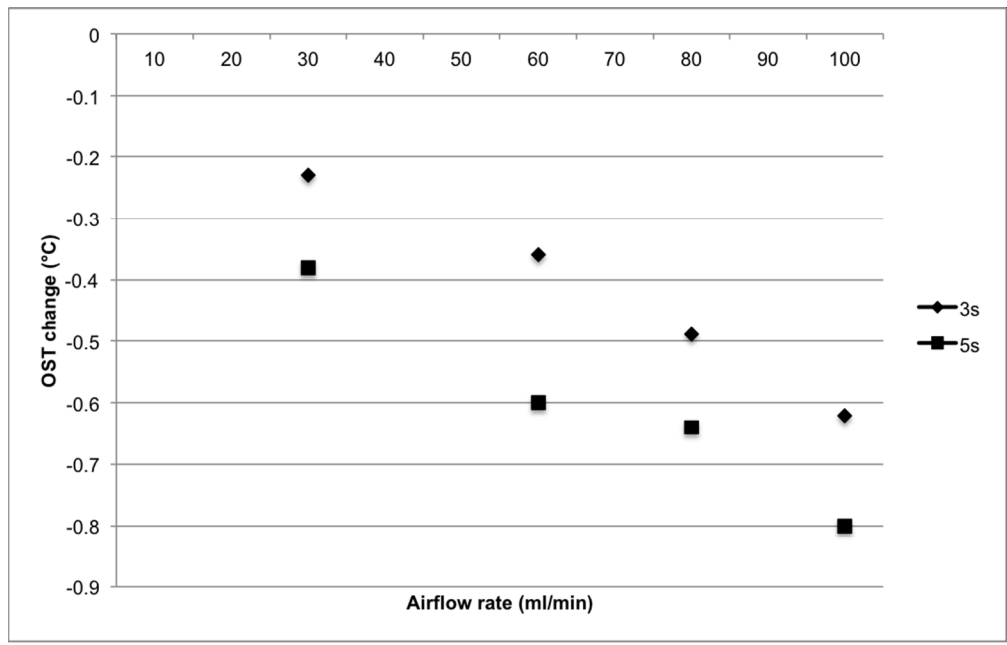
Figure 4: OST change with delta 10
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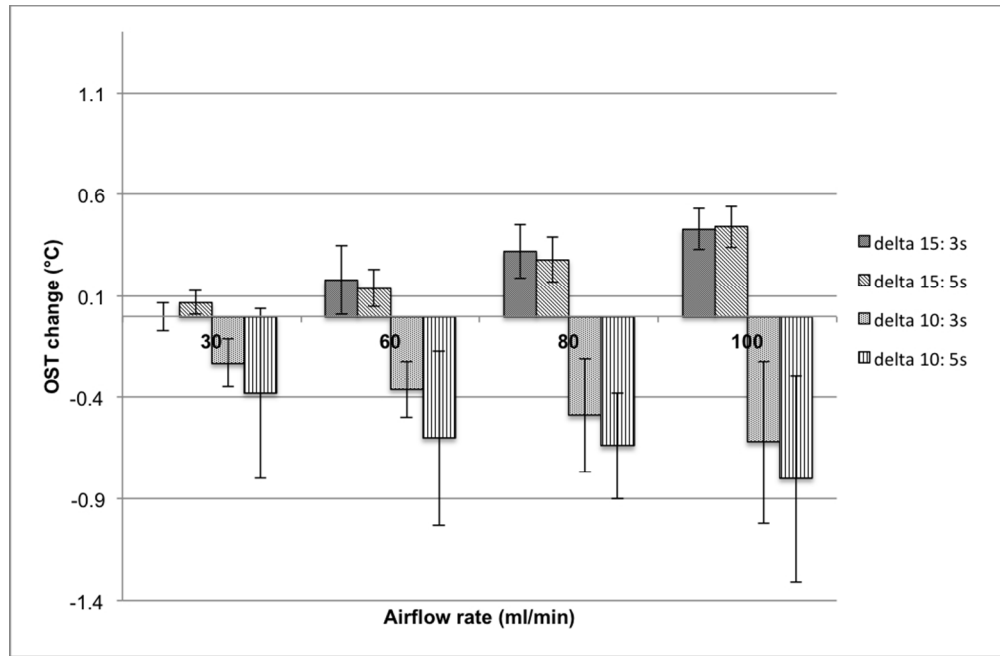
Figure 5: OST change with delta 15

Figure 6: OST change with delta 10 and 15: increase with delta 15 (bars above the x-axis) and decrease with delta 10 (bars below the x-axis).
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