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

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Clinical and Experimental Optometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>Draft</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Original Research Paper</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>n/a</td>
</tr>
</tbody>
</table>
| Complete List of Authors: | Nosch, Daniela; FHNW, University of Applied Sciences, Department of Engineering, School of Optometry  
Pult, Heiko; Optometry and Vision Research; Cardiff University, School of Optometry & Vision Sciences  
Albon, Julie; Cardiff University, School of Optometry & Vision Sciences; Cardiff Institute of Tissue Engineering and Repair  
Purslow, Christine; Cardiff University, School of Optometry & Vision Sciences; University of Plymouth, Faculty of Health and Human Sciences  
Murphy, Paul; University of Waterloo Faculty of Applied Health Sciences, School of Optometry & Vision Science |
| Keywords: | cornea, air gas aesthesiometry, corneal sensitivity, mechanical stimulus |
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 CO₂ 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 Wolffsohn⁴ 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). 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, and in 1999, Belmonte et al. also used 50°C. 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) 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 and tear film stability 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; 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, showing a typical increase of 0.15 to 0.2°C per 1°C increase in room temperature. 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.
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. 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.
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.\textsuperscript{14} A stimulus duration of 3s was
recommended by the manufacturer and was used by Gallar et al.\textsuperscript{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. 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.

<table>
<thead>
<tr>
<th>Delta</th>
<th>Mean OST at baseline ± standard deviation (°C)</th>
<th>Mean OST with stimulus ± standard deviation (°C)</th>
<th>Mean OST change ± standard deviation (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>35.23±0.68</td>
<td>34.26±0.49</td>
<td>-0.97±0.30</td>
</tr>
<tr>
<td>10</td>
<td>35.30±0.80</td>
<td>35.10±0.70</td>
<td>-0.20±0.13</td>
</tr>
<tr>
<td>15</td>
<td>36.24±0.45</td>
<td>36.32±0.42</td>
<td>+0.08±0.05</td>
</tr>
<tr>
<td>20</td>
<td>36.52±0.57</td>
<td>37.28±0.58</td>
<td>+0.77±0.43</td>
</tr>
<tr>
<td>30</td>
<td>36.98±0.53</td>
<td>39.09±1.17</td>
<td>+2.11±0.82</td>
</tr>
</tbody>
</table>
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).

<table>
<thead>
<tr>
<th>Airflow rate (ml/min)</th>
<th>OST at baseline ± standard deviation (°C)</th>
<th>OST with stimulus ± standard deviation (°C)</th>
<th>OST change ± standard deviation (°C)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>34.80±0.85</td>
<td>34.60±0.86</td>
<td>-0.23±0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>60</td>
<td>34.61±0.81</td>
<td>34.22±0.87</td>
<td>-0.36±0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>80</td>
<td>34.73±0.91</td>
<td>34.17±1.03</td>
<td>-0.49±0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100</td>
<td>34.67±0.90</td>
<td>33.96±1.14</td>
<td>-0.62±0.40</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Airflow rate (ml/min)</th>
<th>OST at baseline ± standard deviation (°C)</th>
<th>OST with stimulus ± standard deviation (°C)</th>
<th>OST change ± standard deviation (°C)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>34.50±0.48</td>
<td>34.15±0.51</td>
<td>-0.35±0.42</td>
<td>0.456</td>
</tr>
<tr>
<td>60</td>
<td>34.57±0.54</td>
<td>34.07±0.42</td>
<td>-0.60±0.43</td>
<td>0.027</td>
</tr>
<tr>
<td>80</td>
<td>34.57±0.55</td>
<td>34.07±0.51</td>
<td>-0.64±0.26</td>
<td>0.006</td>
</tr>
<tr>
<td>100</td>
<td>34.63±0.64</td>
<td>33.98±0.63</td>
<td>-0.80±0.51</td>
<td>0.004</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Airflow rate (ml/min)</th>
<th>OST at baseline ± standard deviation (°C)</th>
<th>OST with stimulus ± standard deviation (°C)</th>
<th>OST change ± standard deviation (°C)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>35.24±0.79</td>
<td>35.24±0.78</td>
<td>0.00±0.07</td>
<td>1.000</td>
</tr>
<tr>
<td>60</td>
<td>35.09±0.75</td>
<td>35.27±0.75</td>
<td>0.18±0.17</td>
<td>0.002</td>
</tr>
<tr>
<td>80</td>
<td>35.16±0.82</td>
<td>35.47±0.83</td>
<td>0.32±0.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100</td>
<td>35.17±0.87</td>
<td>35.59±0.92</td>
<td>0.43±0.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Airflow rate (ml/min)</th>
<th>OST at baseline ± standard deviation (°C)</th>
<th>OST with stimulus ± standard deviation (°C)</th>
<th>OST change ± standard deviation (°C)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>34.74±0.56</td>
<td>34.81±0.56</td>
<td>0.07±0.06</td>
<td>0.012</td>
</tr>
<tr>
<td>60</td>
<td>34.83±0.53</td>
<td>34.97±0.54</td>
<td>0.14±0.09</td>
<td>0.003</td>
</tr>
<tr>
<td>80</td>
<td>34.91±0.49</td>
<td>35.19±0.47</td>
<td>0.28±0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100</td>
<td>34.90±0.55</td>
<td>35.34±0.54</td>
<td>0.44±0.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
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 ($\alpha=0.05$; $n=14$; G*Power 3.1):

For airflow rate of 60ml/min:
- OST difference of $0.18 \pm 0.17 ^\circ C$; effect size = 1.059; power = 0.95

For airflow rate of 80ml/min:
- OST difference of $0.32 \pm 0.13 ^\circ C$; effect size = 2.46; power = 1.0

For airflow rate of 100ml/min:
- OST difference of $0.43 \pm 0.10 ^\circ C$; 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.

Delta 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, and the smallest OST difference to be sensed by the ocular surface in humans as 0.3°C. 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, 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


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


Figure 1: Example of localised OST decrease during a cooling air stimulus before local auto adjustment of the area of interest.
Figure 2: Example of localised OST decrease during a cooling air stimulus after local auto adjustment of the area of interest.

430x303mm (72 x 72 DPI)
Figure 3: Localised OST change with increasing levels of delta.

manuscript

160x94mm (72 x 72 DPI)
Figure 4: OST change with delta 10
Figure 5: OST change with delta 15 manuscript
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).