DYNAMIC OCULAR THERMOGRAPHY

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Doctor of Philosophy

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Summary

The ability to measure ocular surface temperature (OST) with thermal imaging offers potential insight into ocular physiology that has been acknowledged in the literature. However, previous technology using cooled detectors has limited the application due to restricted spatial and temporal resolution. The ocular surface has dynamic thermal properties due to the tear film, and therefore static measurements provide limited information: the thermal gradients across the eye's surface are small and ever-changing with each blink.

The TH7102MX thermo-camera (NEC San-ei, Japan) continuously records dynamic information about OST without sacrificing spatial resolution. Using purpose-designed image analysis software, it was possible to select and quantify the principal components of absolute temperature values and the magnitude plus rate of temperature change that followed blinking. The technique was examined for repeatability, reproducibility and the effects of extrinsic factors: a suitable experimental protocol was thus developed.

The precise source of the measured thermal radiation has previously been subject to dispute: in this thesis, the results of a study examining the relationships between physical parameters of the anterior eye and OST, confirmed a principal role for the tear film in OST.

The dynamic changes in OST were studied in a large group of young subjects: quantifying the post-blink changes in temperature with time also established a role for tear flow dynamics in OST.

Using dynamic thermography, the effects of hydrogel contact lens wear on OST were investigated: a model eye for *in vitro* work, and both neophyte and adapted contact lens wearers for *in vivo* studies. Significantly greater OST was observed in contact lens wearers, particularly with silicone hydrogel lenses compared to etafilcon A, and tended to be greatest when lenses had been worn continuously. This finding is important to understanding the ocular response to contact lens wear.

In a group of normal subjects, dynamic thermography appeared to measure the ocular response to the application of artificial tear drops: this may prove to be a significant research and clinical tool.

Key words: Temperature, ocular, contact lens, tear film, artificial tears

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This thesis is dedicated to my husband Steve,
and to our children, Jennie and Jonathan Clewes

- their understanding and encouragement
has been endless.

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INTRODUCTION & LITERATURE REVIEW

The ability to measure the temperature of ocular structures has potential importance in research (Yang and Yang, 1992; Jones, 1998) and ultimately, clinical situations, including ocular physiology (Raflo et al., 1982; Craig et al., 2000), pathology (Keeney and Guibor, 1970; Morgan et al., 1999), ocular blood flow (Auker et al., 1982; Gugleta et al., 1999), contact lenses (Hill and Leighton, 1965a, 1965b; Fatt and Chaston, 1980), and tear film assessment (Morgan et al., 1995; Mori et al., 1997). Measurement of eye temperature has been a technology-driven area of research, most recently with the development of non-contact methods of ocular temperature measurement.

1.1 Evolution in the measurement of body temperature

Heat, as a property of the human body, has been of interest to scientists and clinicians for over two thousand years: in 400BC, the Greek physician, Hippocrates wrote, *"In whatever part of the body excess of heat or cold is felt, the disease is there to be discovered" (Adams, 1939).* Temperature is one of the fundamental parameters of tissue metabolism: all metabolic events within the body produce heat and the heat must be dissipated. Heat is transferred to the body surface by conduction and convection (principally through the vascular system), and radiated to the surrounding environment. Hippocrates was also possibly the first clinician to obtain a thermal image or 'thermogram': he covered a patient's thorax in an earth soaked cloth and the area that dried most quickly was considered the diseased tissue, as it indicated a warmer region (Otsuka and Togawa, 1997).

Ancient civilizations did not attempt to quantify temperature in anything other than a relative way: the technique of using hands to assess temperature remained until the 17th century (Table 1.1).

Table 1.1
Early methods of temperature measurement (after Ring, 1998)

Around 1600, Galileo, the Italian physicist, made a thermoscope made from a glass tube. In 1720, Fahrenheit sealed a similar tube and fixed the scale with the lower point at 0ºF, represented by ice water with salt and the higher point at 212 º F, represented by boiling water. Universally, it is the decimal scale attributed to Celsius (1742) that is used where water boils at 100ºC and ice melts at 0ºC. Interestingly, it was actually Linnaeus (1750) who set the scale this way – Celsius originally designed it in reverse. Professor Carl Wunderlich advanced the use of thermometry in medicine in 1868 with the design of the present day clinical thermometer – mercury in a narrow glass tube, with a kink in the vacuum bore to prevent the mercury running back unless shaken, and a narrow temperature range of 95-105ºF. The mercury thermometer has remained the device of choice for temperature measurement throughout the world. However, in 'developed' countries, it is gradually being replaced by thermocouples and radiometers for middle ear temperature measurement, due to concerns about mercury poisoning and sterility (Ring, 1998).

Another contact method of temperature measurement is *liquid crystal thermography*. Lehmann invented the technique in 1877, when he discovered that some cholesteric esters react to temperature changes by changing colour. 100 years later technology enabled the crystals to be encapsulated into plastic or rubber sheets and applied to the body, resulting in a colour-coded image or thermograph. Clinical use is limited due to its:

- low thermal resolution (±0.5ºC)
- low spatial resolution (±5mm)
- slow response time (>60 sec)
- limited lifespan of the sheets
- subjectivity of interpreting results
- influence on the temperature being measured due to surface contact

(Anbar, 1998)

Liquid crystal thermography has largely been abandoned apart from the Feverscan® forehead thermometer for children.

All these methods rely on contact between device and subject, thereby measuring conducted heat. Non-contact methods include detecting heat convection using Schlerian photography – imaging of the currents surrounding the body, used mainly in the study of insulated clothing (Ring, 1998). Measuring radiated heat is the main method of non-contact temperature measurement (Figure 1.1).

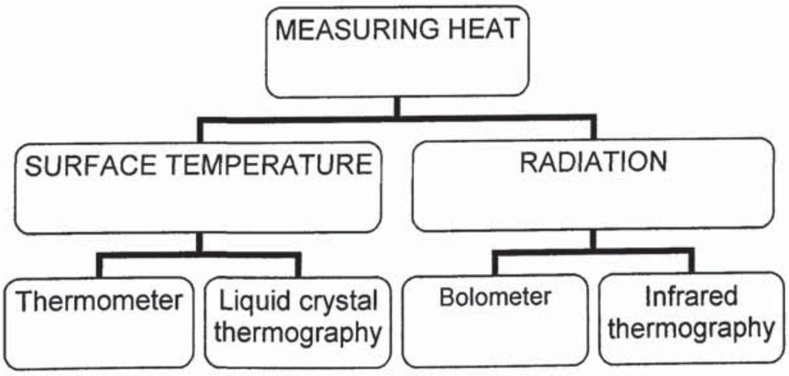

Figure 1.1
Summary of methods of body temperature measurement

1.2 Development of infrared imaging in medicine

The major advances in temperature measurement over the past forty years have been in detecting radiated heat from the body surface. As a non-contact technique, there are some obvious advantages:

- less invasive; more patient-friendly
- potential to be more accurate due to its passive nature
- ability to measure temperature over an area rather than at a single point (this would tend to compensate for local variations in capillary tone)

Radiated heat from the human body is not visible, being part of the infrared region of the electromagnetic spectrum (Figure 2). Astronomer William Herschel discovered infrared radiation in 1800 – he was attempting to determine the heat of different parts of the visible spectrum and discovered that the highest temperature actually fell beyond the red. He went on to show that infrared radiation behaved in a similar way to visible light, in that it could be reflected and refracted. His son, Sir John Herschel recorded infrared radiation by creating an evapograph image using a carbon suspension in alcohol. He called this image a 'thermogram'. Infrared wavelengths range from 75-100,000 x 10^{-6}cm (0.75micron to 1mm; Figure 1.2).

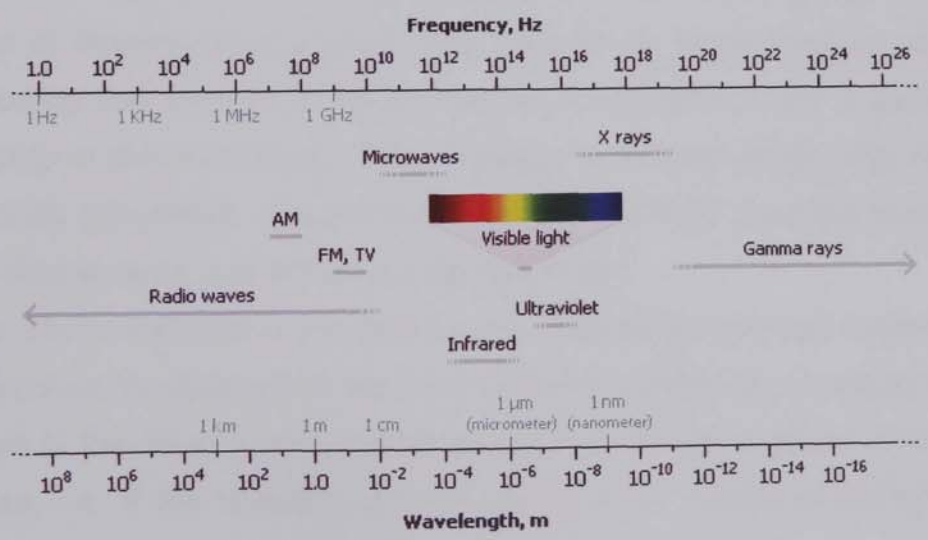

Figure 1.2
The electromagnetic spectrum

Infrared radiation is produced by the motion of atoms and molecules - the hotter an object the more the atoms move, and the more radiation is produced. All objects with a temperature above absolute zero emit infrared radiation from their surface. As the temperature rises, the wavelengths emitted shift towards shorter values, towards visible light, where an object will eventually glow 'red' or be 'white-hot'.

19th century scientists went on to define the relationship between the temperature of the object and the infrared radiation emitted using *black body principles*: when a body at a given temperature radiates energy from its surface, the condition and the colour of the surface are indicative of emitted temperature - a silver, shiny object will absorb less energy than a matt, black surface. An object that is a good emitter must also be a good absorber, otherwise it would eventually radiate all its energy and reach absolute zero - an example of this is the wearing of black clothing in hot climates to keep cool.

The definition of a 'black body' is one that radiates the maximum possible energy at a certain temperature - a perfect radiator, (and also, therefore, a perfect absorber due to thermal equilibrium). In practical terms, it consists of a cavity in the form of a hollow sphere, the inside of which is matt black. This is the standard to which all other surfaces are compared and Planck's Law defines the spectral distribution of radiation from a black body. All ordinary bodies will fall short of this ideal, including skin and the ocular surface, so a correction factor is applied called the *emissivity* of the object/body. The emissivity is defined as the ratio of radiation from the body concerned, compared to the radiation from a perfect black body at the same temperature, and will always be less than 1.

The *Stefan-Boltzmann law* defines the relationship between radiated energy and temperature by stating that the total radiation emitted by an object is directly proportional to the object's area and emissivity and the fourth power of its absolute temperature, i.e. *if the radiation and emissivity of an object is known then the temperature can be calculated* (Lindsay, 1971; Jones, 1988).

Technological innovations of the 20th century allowed progress beyond the theoretical stages. Hardy devised one of the first infrared detectors for skin temperature measurement (Hardy, 1934), but the important advances in infrared imaging were made by military scientists for use in surveillance, night-vision devices, heat-seeking missiles, etc. When American and British governments in the 1950s declassified the principles behind their technology, medical scientists began to investigate their potential application in body temperature measurement. Research in the 1950's and 60's adapted these ex-military prototypes for clinical use in neurology, surgery, oncology, dentistry and dermatology. For instance, in the UK, medical scientists used an ex-military system to image patients with arthritis as early as 1959 (Ring, 1998), whilst others examined patients with tumours, cysts and vascular disease (Lloyd Williams et al., 1960). However, although the potential of this non-invasive technique was acknowledged at the time, infrared cameras were inadequate, leading to some early work in the field being discredited (Diakides, 1998). The technology at that time was limited by:

- Poor spatial resolution, typically 128 x 128 pixels
- Limited thermal resolution
- Slow scanning times, often requiring 4 seconds or more, further reducing spatial and thermal resolution
- Calibration difficulties; lack of standardized black bodies
- Inappropriate choice of detector material - cameras using InSb (Indium Antomide) detect in the range 3-5 micron, but in this region skin is less like a perfect radiator and reflects 10-15% (Anbar, 1998)

In the 1990's space exploration and astronomical observations held major objectives for infrared imaging, resulting in the development of high-performance infrared detection systems, now used in many industries.

A typical infrared-measuring device consists of a system for collecting radiation from a defined field-of-view: in practice this is a coated lens system (Germanium) designed to allow a specific portion of the infrared spectrum to pass through to a photo-sensitive detector material. The detector converts the infrared energy into a measurable voltage, current or resistance. The detectors spectral sensitivity in combination with that of the optical system determines the system's spectral response. Three types of detectors commonly used are *photon* (release electrical energy in response to incident radiation), *pyroelectric* (respond to changes in incident radiation and require 'choppers' to modulate incident radiation and maximise sensitivity) and *thermovoltaic* (provide a voltage in response to incident radiation). First generation systems used a scanning system of detectors, but the latest generation use a focal plane array which not only gives improved performance and facilitates digital output, but does not require cooling by liquid nitrogen. An amplifier increases the level of detector signal for subsequent processing, and then electronic circuits process the measured data via computer software. All infrared detectors need to be calibrated against a known perfect radiator (a black body): early models needed an external source to do this, but the latest instruments are self-calibrating. Early devices commonly displayed the signal from the detector as a reading on a dial (bolometer or radiometer), but image processing is now widely used to display a thermal picture on a monitor: termed *thermography*. These features have been integrated into high-performance IR systems, with typical specifications:

- thermal resolutions of 0.1°C;
- accuracy of ±2%;
- images of 256 x 256 pixels or greater;
- frame refresh rates of >10Hz;
- spatial resolutions of up to 50μm
- self-calibration to black body standards
- spectral ranges of 8-14μm (thereby reducing the effect of reflected solar radiation)

Medical thermography has a large bibliography (Jones, 1998) and the technique has been used to investigate a wide variety of conditions. Much of its medical use is based on the detection of angiogenesis in tumours, and abnormalities in blood flow to inflamed areas. An understanding of the mechanism of thermal behaviour is key to the optimal use of thermal imaging in clinical diseases (Ring, 1998), oncology (especially breast cancer (Keyserling et al., 1998), dentistry (Hussey et al., 1997; McCullagh et al., 1997; Gratt and Anbar, 1998) and ophthalmology (Raflo et al., 1982).

In abnormalities of the female breast, malignant tumours tend to be warmer than benign tumours due to increased metabolism and vascular changes surrounding the tumour, and the technique has been recommended as a useful adjunct to mammography (Keyserling et al., 1998). It has had FDA approval since 1982, and studies have demonstrated high sensitivity and specificity (Nyirjesy and Ayme, 1986).

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In peripheral vascular disease, it has been suggested that the ideal investigative pathway for deep vein thrombosis should thermography prior to ultrasonography, as it is non-invasive, risk-free and quick (Harding, 1998). Thermography has also been used to investigate the effects of smoking on peripheral vasoconstriction (Fushimi et al., 1998).

In musculoskeletal disease, thermography is able to distinguish between deep inflammation and more cutaneous involvement in arthritis (Ring, 1998).

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(Photos taken from www.meditherm.com)

In dentistry, thermography has been used to assess the frictional heat effect during various treatments (Hussey et al., 1997; McCullagh et al., 1997), and to aid understanding of oral-facial pain (Gratt and Anbar, 1998).

Body temperature measurements require knowledge of the emissive properties of the surface being examined. It has been established by different groups of workers that the surface of the skin (irrespective of colour) is an efficient radiator and approximates to a black body with an emissivity of 0.98±0.19 (Steketee, 1973). Skin temperature is normally maintained within the range 25-35^{0}C (around 300K), and over this range emission occurs between 2 and 50μm with maximum emission of 9-10μm (Figure 3). It has also been shown (Steketee, 1973b) that infrared radiation from deeper tissues is not transmitted for wavelengths greater than 1.2 microns, or vessels deeper than 7mm (Jones, 1988).

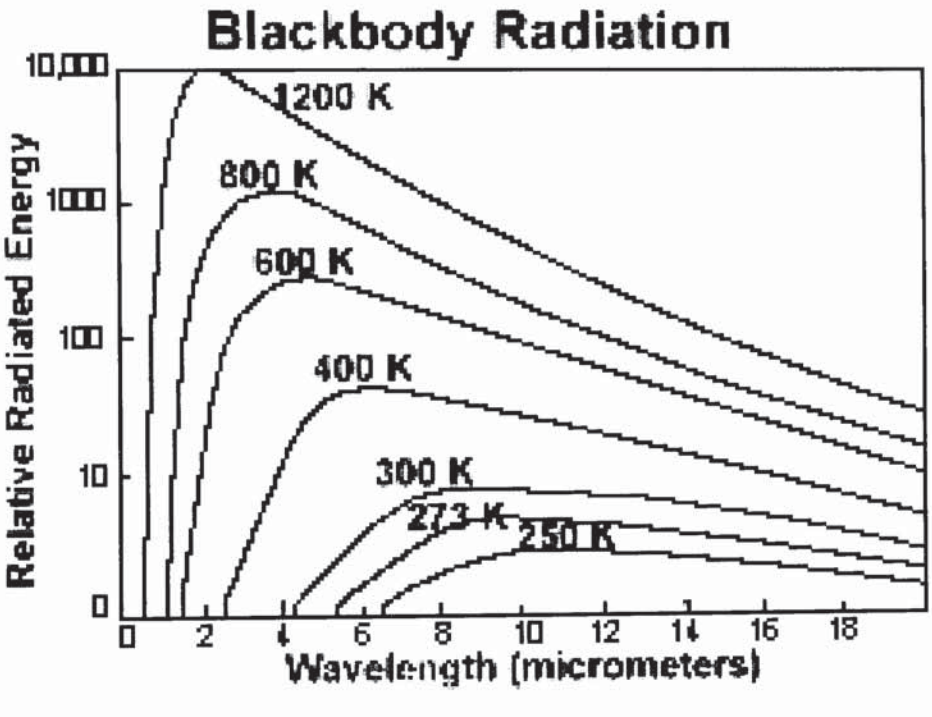

Figure 1.3
Emission spectrum of a black body at various temperatures

There is evidence of increasing use of *dynamic* infrared thermography in medicine which is a more powerful clinical tool than thermal imaging at a single time (Anbar, 1994). For example, sensitivity can be increased by the use of cold stress techniques applied to the female breast: the thermal recovery pattern can be time (Anbar, 1994). For example, sensitivity can be increased by the use of cold stress techniques applied to the female breast: the thermal recovery pattern can be seen dynamically when the stress is removed (Ohashi and Uchida, 2000). This information appears more useful to the clinician.

Applications for thermography outside medicine include quality assurance, plant maintenance, automotive, aviation, chemical, civil engineering and construction industries, as well as research and development.

1.3 Thermometry and the Eye

As one might expect, the developments in ocular thermometry have reflected the general advances in temperature measurement.

1.3.1 Contact methods of ocular thermometry

The first one hundred years of labours surrounding ocular temperature measurement using contact techniques are summarised in Table 1.2.

YEAR	*AUTHOR(S)*	*METHODOLOGY*	*EYE TEMP (°C)/ FINDINGS*
1875*	Dohnberg	Specially adapted glass mercury thermometer placed in *conjunctival sac*. Observed higher temperatures in acute iritis.	36.5-36.7 (norm)
1877*	Galezowski	Mercury thermometer in normal and inflamed eyes.	36.4 (normal) 36.9-37.1 (inflamed)
1893*	Silex	Thermo-element in the inferior fornix. Temps in acute iritis higher.	35.55 (normal) +1.56 in iritis
1894*	Giese	Thermo-element in the inferior fornix	35.72 (normal) 38.19 (iritis)
1900*	Hertel	Mercury thermometer	35.65 (normal) 36.95 (iritis)
1913*	Howe	Compared eye temperature with oral temperature	0.3-0.4°C lower than oral temperature
1942*	Kirisawa	0.2mm & 1.0mm thickness of thermo-element in conjunctiva	36.34 & 34.50
1952	(Holmberg, 1952)	0.2mm thermo-element in conjunctiva	2.5°C lower than oral temperature
1950**	Stoll & Hardy	Contact method	Observations of ocular temperature
1952**	Braendstrup	Contact	Observations of ocular temperature
1962**	Schwartz & Feller	Contact	Observations of ocular temperature
1965	(Hill and Leighton, 1965a, 1965b)	Thermistor attached to scleral contact lens	Observations of changes in tear film temp behind scleral lens

1965	(Schwartz, 1965)	Thermistor in hypodermic needle inserted in eyes of rabbits	Demonstrated ocular gradients in rabbits
1970	(Kolstad, 1970)	Thermistor in glass probe placed directly on anaesthetized cornea	Observations of corneal sensitivity at low temps.
1972	(Fatt and Forester, 1972)	Thermistor in metallic probe inserted into agar jelly and rabbit eyes	Demonstrated the errors that can arise when using a metallic probe.
1973	(Freeman and Fatt, 1973)	Thermistor inserted into rabbit eyes to measure surface and intra-corneal temperature	Observations of effect of environment on eye temperature in rabbits
1973	(Kinn and Tell, 1973)	Liquid crystal layer in scleral contact lens which is heat-sensitive	Measurements of surface temperature
1975	(Horven, 1975)	Thermistor probe placed on cornea	Corneal surface temperature in normals and in eye disease
1977	(Rosenbluth and Fatt, 1977)	Fine-wire thermocouple inserted into rabbit eyes	Exploring sources of error in this contact method
1982	(Auker et al., 1982)	Flat, circular thermistor probe	Scleral & conjunctival temperature assessed as a measure of choroidal blood flow
1985	(Holden and Sweeney, 1985)	Thermistors embedded in a ring to sit in palpebral aperture	Measured oxygen tension and temperature of the upper palpebral conjunctiva
1986	(Martin and Fatt, 1986)	Thermistors sandwiched between soft contact lenses	Observed small increases behind the contact lenses

Table 1.2
Contact methods of ocular thermometry
*recorded by Holmberg (Holmberg, 1952)
**cited by Mapstone (Mapstone, 1968a)

The results amongst the contact studies demonstrate large variance; probably due to a combination of the differing methods employed, which part of the eye was examined, whether anaesthetic was employed and the surrounding environment. These contact, sometimes invasive techniques have some obvious disadvantages:

1) Contact lens methods (Hill and Leighton, 1965a, 1965b; Kinn and Tell, 1973) are clearly not representative of the ocular surface in air, and complex modelling would be required to determine any correction factors. The colour responses from

Kinn & Tell's liquid crystal lens device were also difficult to interpret, mostly due to poor resolution.

2) Where the cornea is perforated, the work is obviously limited to animals, and even if with minimal contact, there is a possibility of trauma.

3) The use of topical anaesthetics influences the temperature recorded due to the temperature of the drug itself and the reflex tearing its instillation induces.

4) With any probes containing a thermistor, the actual temperature measured is somewhere between that of the air and the cornea, as it is exposed to both. Even when the air-exposed part of the probe is sheathed, temperature gradients still exist along the probe (Fatt and Forester, 1972; Rosenbluth and Fatt, 1977).

5) It has also been demonstrated that the probe acts as a cooling fin, increasing the available surface area for heat conduction away from the eye, particularly when the probe is used at minimal depth, or when large gauge needles are used (Fatt and Forester, 1972); (Rosenbluth and Fatt, 1977). This has the effect of recorded temperatures being actually lower than the true corneal temperature.

6) All of these methods only measure temperature at one specific point at any one time, of limited value considering the intrinsic haemodynamics and temperature gradient across the eye (Efron et al., 1989).

7) Contact measurements of temperature vary with the pressure of application of the probe (Mapstone, 1968a).

However, thermistors have continued to be used by some researchers especially with animal work and in work involving contact lenses. Auker (Auker et al., 1982) used a circular, flat thermistor on cats and monkeys to show that scleral and conjunctival temperature could be used as a measure of choroidal blood flow; previously this comparison had been made using intra-ocular temperatures. Holden and Sweeney used thermistors and oxygen sensors embedded in a ring designed to fit into the human palpebral aperture to directly measure the oxygen tension and temperature of the upper palpebral conjunctiva, representing closed eye conditions (Holden and Sweeney, 1985). Martin and Fatt attempted confirmed heat transfer modelling using thermistors between thin contact lenses to measure heat exchange at the cornea-contact lens interface (Martin and Fatt, 1986).

1.3.2. Non-contact methods of ocular thermometry

Contact thermometry of the human eye has largely been rejected in favour of the more practical and, potentially, more accurate techniques of non-contact infrared radiometry. A radiometric method was first employed by Zeiss in 1930 (Mapstone, 1968a), but the technique was developed by Mapstone in the 1960s and presented in a series of four articles (Mapstone, 1968a, 1968b, 1968c, 1968d). A *bolometer* (Figure 1.4) was used, employing the principle that infrared radiation incident upon an electrical conductor produces a change in resistance (through a change in temperature), which can be amplified and converted directly into a temperature reading. It was sensitive to infrared radiation in the range 1-25μm and was calibrated against a black body kept at a constant temperature. The bolometer was positioned 5mm from the cornea (lids retracted if necessary), so that the area measured had a diameter of 10mm. Measurements were taken between blinks, and after 15 minutes of room adaptation, where the room was kept between $18^{\circ}C$ and $26^{\circ}C$.

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Figure 1.4
Mapstone's bolometer set-up (Mapstone, 1968b)

To convert radiation readings to true temperatures, it is necessary to know how close the tear film and cornea are to the black body ideal. If the transmission/absorption characteristics of the ocular tissues are known then the radiation characteristics can be deduced by Kirchoff's Law [Kirchoff's law states that the absorption and emission characteristics of a body are equal at any given temperature (Lindsay, 1971)], and the application of the Stefan-Boltzmann equation (section 1.2).

Water is an effective absorber of infrared radiation (Lerman, 1980) and it is logical that the high water content of tears, cornea and lens should ensure high absorption characteristics. The cornea transmits visible radiation, but at the 1.4μm infrared level, transmission begins to fall off rapidly. Above 1.8μm, transmission is only a few percent and is absent above 2.3μm (Lerman, 1980). Thus, the cornea can be considered an efficient *absorber (and therefore, radiator)* of infrared radiation in this portion of the spectrum, and to have radiation *emissivity* close to that of a black body (Rysa and Sarvaranta, 1974). For example, if the radiation emitted by the eye has a spectrum curve between 1 and 30μm with a peak at 9μm, then the black body that has a similar curve is known to be at 32°C (Figure 1.5).

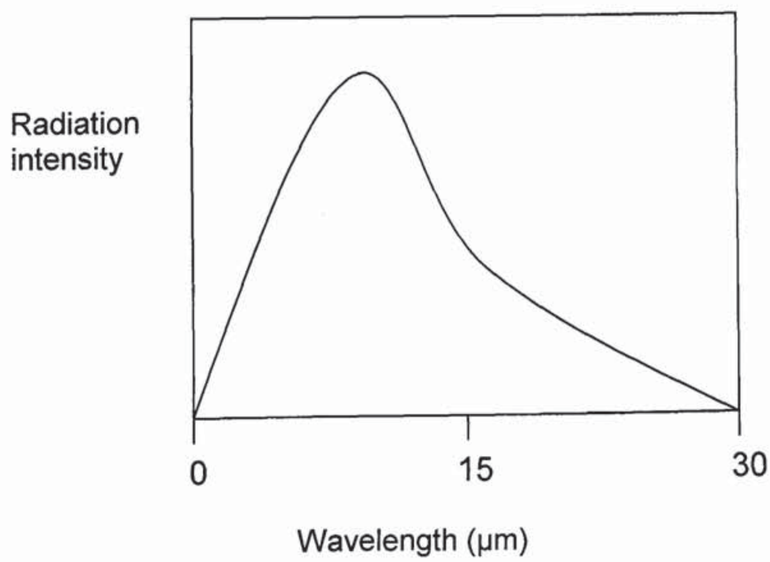

Figure 1.5
Emission spectrum of black body at 32°C (peak at 9μm)

Exactly how close the cornea is to a perfect black body is important: Mapstone reasoned that the true temperature of the cornea ought to be same as the body core (37^{0}C) and therefore if the Stefan-Boltzmann equation is applied, the cornea would have an emissivity of 0.97. As the cornea will be generally cooler than body temperature, the emissivity will actually lie between 0.97 and 1 (Mapstone, 1968a). An emissivity value of 0.98 is generally accepted by scientists for both the skin and ocular surface.

Mapstone also recognised that the tear film must play an important role in the measured temperature: he regarded the tear film and cornea as one continuous water phase, with both behaving as black bodies. He suggested that the bolometer recorded a transient equilibrium between the tear film and cornea, where heat exchange would take place following a blink to restore this equilibrium. It has been established that the media of the human eye absorb and emit infrared radiation efficiently, in a similar way to water (van den Berg and Spekreijse, 1997); and the tear film, with its high water content, should also be an efficient absorber/radiator. Using the data from Hamano (Hamano et al., 1969), it can be calculated that infrared radiation from the cornea will be completely absorbed by a tear film thickness of 40µm (Prydal et al., 1992). However, tear film thickness is still under debate (Craig, 2002): if the minimum values are selected from research (King-Smith et al., 2000), Hamano's work suggests an absorption of around 80% (Figure 1.6).

Figure 1.6
Percentage absorption of radiation between 7.5-15μm by increasing thickness of water (taken from Hamano et al, 1969)

Further studies have therefore suggested that the measured temperature is essentially that of the tears (Hamano et al., 1969; Fatt and Chaston, 1980; Morgan et al., 1993), and only where the tears are absent will the radiation detected be that from the cornea itself. The tear film is a dynamic structure and changes in its thickness, composition and evaporation rate will alter the measured temperature. In view of the current dispute over tear film thickness, It is perhaps more reasonable to predict that as the tear film decreases in thickness, the posterior structures, i.e. the cornea, will have increasing contribution to the radiated temperature that is measured.

Mapstone considered the deeper ocular structures to contribute little to the emergent radiation from the anterior eye: all wavelengths above 3μm radiated by posterior tissues will be absorbed by the lens, cornea and tear film, leaving only a small portion to effectively contribute. The vitreous is opaque to radiation beyond 1.4μm. However, that is not to say that measured radiation is unaffected by its surroundings; it will be influenced by internal and external factors (Wachtmeister, 1970).

These factors include:

- Heat transfer from adjacent structures by conduction and convection (Mapstone, 1970).
- Blinking spreading warm tears over the cornea and conjunctiva (Mapstone, 1968b).
- The relatively cooler environment will cause the surface to cool in an effort to maintain equilibrium (Wachtmeister, 1970; Serway and Beichner, 2000).
- As tears thin post-blink, the surface will appear to cool (Morgan et al., 1995; Craig et al., 2000). It has been shown that rapid cooling of the tear film in dry eye conditions appears to be related to reduced tear film stability (Craig, 1997) and the increased rate of evaporation.
- Changes in blood flow to the eye (Mapstone, 1970) or head (Mapstone, 1968b; Cardona et al., 1996; Morgan et al., 1999).

It is perhaps more appropriate to call the radiated temperature *Ocular Surface Temperature* (OST), rather than assign it to any particular structure in the anterior eye. In most studies it is the OST in the central area, overlying the cornea and limbal areas, that has been the main focus. In his PhD thesis, Morgan referred frequently to MOST - Mean OST (Morgan, 1994) .

Table 1.3 displays a summary of research using infrared ocular thermometry from the 1968-88 period, including Mapstone's work. These methods still had the disadvantages of being very close to the eye (inducing reflex tearing), and only recording temperatures over a small corneal area.

Table 1.3: Early studies using infrared thermometry

Author & Date	APPLICATION	METHOD	Subjects	CONCLUSIONS
(Mapstone, 1968a)	To measure normal corneal temperature	Bolometer - subject room adapted for 15mins; late afternoon; room temperature 18-26°C; lids retracted; blink between measures; area measured 10mm in diameter	140 normal corneas from 70 subjects	Mean temp 34.8°C Range 33.2-36.0°C
(Mapstone, 1968b)	To examine factors that determine corneal temperature and cause differences between the eyes	Bolometer - as above plus 1. Lids closed for 5mins to assess effect of 2. Lids retracted for 5mins with topical anaesthetic 3. Irrigation with and without blinking allowed 4. 4 Subjects with unilateral anterior uveitis 5. 5 Subjects with carotid artery stenosis 6. 4 Subjects with malignant choroidal melanoma	1-5 subjects	1. Lid closure causes mean rise of 1.5°C 2. Lid retraction causes mean drop of 1.1°C 3. Blinking helps restore normal corneal temperature 4. Inter-ocular temp differences decline as condition improves 5. Cornea of affected side had lower temperature than that of opposite side. 6. Insignificant difference between affected eye and unaffected eye => *Abnormal temperature differences between two eyes are a response to different aqueous temperatures, not to external or posterior eye factors.*
(Mapstone, 1968c)	To examine normal patterns in cornea and periorbital skin	Bolometer - 15 min room adaptation; late afternoon; room temperature 18-27°C measurements made over four areas - cornea, medial forehead, lateral forehead and lower lid.	70 normal subjects	Normal differences between eyes are no greater than 0.4 and 0.6°C. Normal subjects either have no temperature difference or one side is consistently hotter/colder than the other. Environmental temperature changes affect periorbital areas and cornea by similar amounts.
(Mapstone, 1968d)	To examine thermal patterns in anterior uveitis	Bolometer - as above	53 unselected subjects with unilateral anterior uveitis	An increase in corneal and periorbital temperature is seen on the affected side. A positive correlation between maximum corneal temperature and duration of ciliary injection.

(Schwartz et al., 1968)	To examine the influence of age, sex, race, relative humidity, oral temperature, room illumination and time of day on ocular temperature and forehead temperature	Stoll-Hardy radiometer	131 subjects	Ocular temperature highly correlates with forehead temperature, but independent of all other variables except time of day and age of subject
(Rosenbluth and Fatt, 1977)	To explore the sources of error in using intra-ocular thermocouples on rabbits by comparing readings with those from a Dermo-Therm® unit	Dermo-Therm® unit (similar to Mapstone's bolometer), held 1cm from the cornea; area measuring 3mm in diameter.	Rabbits	Errors exist when using probes due to heat conduction losses from the probe and trauma to the eye
(Fatt and Chaston, 1980)	To look at the mean corneal temperature and compare bare corneas with those covered by hard and soft contact lenses. temperature was also measured after the lens was removed	Dermo-Therm® bolometer – area measuring 4mm in diameter; sensitivity of 0.25°C	6 subjects for bare cornea (25-55 yrs) Another 3 wearing hard lenses, and 3 wearing soft lenses	Bare corneas range 33-36°C Eye wearing hard contact lens (lower thermal conductivity) 0.5-1.5°C below that of same eye without lens Eye wearing soft contact lens never more than 0.5°C below that of same eye without lens
(Efron et al., 1988)	To investigate the relationship between bulbar conjunctival hyperaemia and temperature	C-600M isotherm Non-contact Infrared Bolometer (Linear Lab, USA). Use of hypertonic saline to induce redness.	18 subjects (12M, 6F, age 23±4 years)	Hypertonicity and resulting blepharospasm induce redness and temperature increases. An increase in one grade of hyperaemia corresponded with an increase in temperature of 0.15°C, but inter-subject variability was high precluding the use of a single measurement for diagnostic purposes.

1.3.3 Ocular Thermography

By 1970, Mapstone had progressed to utilize an infrared camera that produced a thermal picture, rather than a reading on a dial. This had the advantages of being entirely non-invasive and allowed the surface thermal pattern over an area to be seen. Measurement and colour-coded display of eye temperature is referred to as *ocular thermography* (Mapstone, 1970).

Since the 1950s, medical thermography has been recognised as a useful clinical tool, but it was not until the 1970s that *ocular* thermography was explored. The reasons for this were largely practical as early infrared cameras could not cope with the small temperature gradients involved, magnification was limited, and scanning times were too long. Table 1.4 summarises previous applications of ocular thermography with these 'first generation' cameras.

Table 1.4: Applications of ocular thermography - first generation cameras.

Author & Date	APPLICATION	METHOD	Subjects	CONCLUSIONS
(Mapstone, 1970)	To examine thermographic patterns in normal, ischaemic and hyperaemic ocular states	Bofors infrared camera system; thermal picture registered on an oscilloscope	30 subjects	Thermal patterns around the eye are described
(Wachtmeister, 1970)	To examine normal eyes and eyes with pathology	Bofors system & AGA Thermovision®	38 subjects with unilateral or bilateral eye disease; 41 healthy subjects	In cases of anterior and posterior eye disease, the affected eye was on average warmer than the fellow eye. It was felt that technology needed to improve before it could become a useful clinical tool.
(Rysa and Sarvaranta, 1973, 1974)	1. Observations of corneal temperature during cold stress 2. Observations of corneal temperature in man and rabbit (who blink less frequently than man)	AGA Thermovision® 680 system	20 subjects 78 rabbit eyes; 12 human eyes	1. A fall in corneal temperature with cold stress may be correlated with the thickness of the cornea and the depth of the anterior chamber 2. Corneal temperature correlates strongly to body temperature in both rabbits and humans; blinking is followed by a rise in temperature; a human cornea cools more rapidly than a rabbit – postulated to be due to larger surface area or possibly shallower anterior chamber?
(Mikesell, 1978)	A study of normal and laser-injured corneas in rabbits	Radiometer from Barnes Engineering Company; measurements taken at baseline and subsequent days after laser insult to cornea; 7 mm diameter are measured.	172 rabbit corneas	Rise in corneal temperature after laser injury observed
(Fielder et al., 1981)	Examined thermographic patterns in relation to corneal arcus	AGA Thermovision® 680 system; purpose-built draught-free room	Unclear	Arcus begins in the warmest part of the eye, and in cases of unilateral arcus, it is the warmer eye that is involved.

(Alio and Padron, 1982a, 1982b)	Examined normal variations in the orbito-ocular region and the influence of age on corneal temperature	AGA Thermovision® 680 system – 102B. Absolute temperatures of five points measured – cornea centre, limbus, sclera, outer and inner canthus.	96 subjects	Asymmetry between the eyes featured in 57% of subjects (difference of 0.51± 0.06°C) – show fallacy of considering thermographic asymmetry as criteria of abnormality. Corneal, limbal, scleral and outer canthus temperatures decrease significantly with age; whereas inner canthus temperatures show very little variation (outer canthus shows most). No significant difference between sexes or sides was found ($P>0.05$) Limbus 0.6°C warmer than corneal centre.
(Coles et al., 1988)	To relate stages of wound healing and inflammatory responses to temperature in rabbits	DRGX thermo-digital camera		Thermograms indicate stages of wound healing and intra-ocular inflammatory responses, in limbal incisions in rabbits.
(Efron et al., 1989)	To explore the corneal thermal profile and the temporal stability of temperature	Thermo Tracer 6T61 (NEC San-ei Instruments, Japan); 6x4.5cm area imaged at 15cm distance from cornea	21 subjects (12M, 6F, aged 31±10 years)	Advent of superior technology Recognition of ellipsoidal isotherms with major axis horizontal, concentric about a temperature apex which is slightly inferior to the geometric corneal centre. Observed decrease in temperature following a blink, possibly due to tear evaporation? Limitations due to inability to provide accurate anatomical localisation, and high cost.

A second generation of infrared cameras was used extensively by Morgan and co-workers at UMIST in the 1990s, amongst other researchers (Table 1.5). The 6T62 Thermo Tracer (NEC San-ei Instruments, Japan) had a Germanium window set before the detector unit that was sensitive to wavelengths 8-13μm. The optical scanning device employed mirrors to scan the surface from top to bottom and left to right: scan times varied between 0.25, 0.5 and 1 second, but spatial resolution suffered at anything faster than 1Hz. The focussing of the system was controlled by adjusting the position of the objective lens. It had a working distance of 40cm, but a close-up lens could be employed to allow a more detailed view: this had a focal range of 37-53mm, and a maximum magnification of up to x30 using zoom facility. A chopper device created an alternating signal to the detector (an alloy of cadmium, mercury and tellurium - CMT) which was cooled by liquid nitrogen. The thermal image was displayed in 8 bit colour coding (256 colours) on a 30cm monitor. Morgan's experiments were conducted within a remote-controlled thermal cubicle containing subject and camera; temperature was recorded from five areas across the ocular surface, and typical thermal sensitivity employed was 0.2°C (Morgan et al., 1993; Morgan, 1994).

Table 1.5: Applications of ocular thermography - second generation cameras.

Author & Date	APPLICATION	METHOD	Subjects	CONCLUSIONS
(Morgan et al., 1993)	To explore the potential applications of ocular thermography	6T62 Thermo Tracer (NEC San-ei Instruments)	98 normals	95% of population have Interocular temperature difference of 0.6°C or less. No significant difference due to race, order of eye measured, gender, age or which eye measured found in this study. Steeper thermal gradients across cornea in dry eye patients observed
(Fujishima et al., 1994)	To quantify post-surgical inflammation in cataract patients	Comparing laser flare-cell meter with infrared radiation thermographer	40 cataract surgery patients	The longer the cataract surgery, the greater the inflammation. Laser flare-cell meter more sensitive: thermography only detects traumatic surgery.
(Fujishima et al., 1995)	To examine the effect of cooling post-cataract surgery on comfort and inflammation	Comparing laser flare-cell meter with infrared radiation thermographer	20 bilateral cataract surgery patients	Cooling significantly decreased corneal temperature and flare, and increased subjective comfort post-operatively
Hata et al, 1995 (Hata et al., 1994)	To examine whether change in humidity and temperature can effect blinking	Infrared radiation thermographer	4 normal subjects	In cool conditions and low humidity, corneal temperature decreased and blink rate increased. Observed that corneal temperature decreases upon eye opening.
(Morgan et al., 1995)	To examine the thermal profile of dry eyes	6T62 Thermo Tracer (NEC San-ei Instruments)	36 dry eye patients & 27 age- and sex-matched controls	Mean ocular temperature greater in dry eye group, with greater variation across cornea. Hyperaemia of a dry eye outweighs increased cooling due to tear evaporation
(Cardona et al., 1996)	To examine the temperature of the eye and facial skin in patients with post herpetic neuralgia	6T62 Thermo Tracer (NEC San-ei Instruments)	12 patients with post-herpetic neuralgia of >5/12 duration	Ocular surface of the affected side was significantly colder, but the skin temperature was not - ocular ischaemia/ sympathetic nervous system upregulation?

(Morgan et al., 1996)	To examine the changes in thermal profile of dry eyes upon eye opening	6T62 Thermo Tracer (NEC San-ei Instruments)	11 dry eye & 7 controls	Significantly faster rate of cooling in dry eye group due to greater evaporation rate and increased initial temperature?
(Betney et al., 1997)	To quantify the temperature changes during PRK	6T62 Thermo Tracer (NEC San-ei Instruments)	12 subjects undergoing PRK	*It is possible for the cornea to reach temperature levels where it is known that proteins denature.* Factors such as ablation depth, optical correction and duration of procedure were not significant to temperature.
(Schrage et al., 1997)	To examine the temperature changes caused by two types of eye bandage	Joel infrared camera	40 normal subjects	Application of an eye bandage increase the corneal temperature significantly - a change in the bacterial spectrum may result. cooling may be better in treating sterile, but inflamed eyes.
(Mori et al., 1997)	To evaluate the tear film of dry eye patients by measuring corneal temperature	Infrared radiation thermographer; measured the rate of temperature change (k value)	13 dry eye patients & 7 normals	Rate of temperature change in dry eye subjects *less* than in normals
(Fujishima et al., 1997)	To examine the effects of artificial tear temperature on corneal sensation and subjective comfort	Infrared radiation thermographer	24 normal subjects	Corneal temperature was significantly lowered with all temperature artificial tears. Corneal and conjunctival sensitivity were inversely correlated with corneal temperature.
(Du Toit et al., 1998)	To measure corneal thickness, sensitivity and temperature over 24 hours	Optical pachometer; pneumatic aesthesiometer	20 subjects	Corneal thickness increased during sleep and then decreased during the day while sensitivity and temperature decreased during sleep. Recovery to baseline was slowest for temperature.
(Girardin et al., 1999)	To examine the relationship between corneal temperature and finger temp	THI-500 infrared thermometer (Tasco, Japan) - corneal and finger temperature measured with IR camera and body temperature measured with ear thermometer.	266 subjects (124F, 142M)	Corneal temperature correlates with finger temperature even after adjusting for environmental temperatures and for age and sex of subjects - parallelism in blood flow regulation to both areas?
(Gugleta et al., 1999)	To study whether arterial blood flow correlates with ocular temperature.	THI-500 infrared thermometer (Tasco, Japan) to measure corneal temperature & colour Doppler imaging (CDI) to measure blood flow in both ophthalmic arteries. 15mins room adaptation	18 normals; 18 glaucoma patients	Suggests that retrobulbar haemodynamics influence corneal temperature, and that this relationships comparable in normal subjects and glaucoma patients. Interocular corneal temperature difference correlated with difference in end diastolic velocity (EDV)

(Kocak et al., 1999)	Assessed the reliability of the technique - reproducibility, Interocular and diurnal changes examined	THI-500 infrared thermometer (Tasco, Japan)	10 healthy subjects	Highly reproducible technique. Interocular differences not statistically significant. Average temperature varied significantly throughout the day (lower in the morning), independent of variations in environmental temperature.
(Morgan et al., 1999)	To explore the potential of thermography in the investigation of carotid artery stenosis	6T62 Thermo Tracer (NEC San-ei Instruments) & colour duplex ultrsonography	24 subjects	Significant negative correlation between ocular temperature and degree of stenosis.
(Murphy et al., 1999)	To investigate whether change in corneal temperature is a component in how the aesthesiometer stimulates the cornea	6T62 Thermo Tracer (NEC San-ei Instruments) & non-contact corneal aesthesiometer	9 subjects; model cornea	The air-pulse stimulus was capable of producing localized temperature reduction, proportional to stimulus pressure. The principal mode of corneal nerve stimulation by the aesthesiometer was the rate of change in corneal temperature.
(Morgan et al., 1999)	To study the relationship between corneal temperature and age	6T62 Thermo Tracer: NEC San-ei Instruments	98 subjects (56M, 42F, age 7-83 years)	Reduction of 0.01°C per year - main cause is probably atherosclerosis/ age-related changes in tear film?
(Maldonado-Codina et al., 2001)	To investigate the temperature changes occurring during PRK when performed at different ablation depths,	6T62 Thermo Tracer (NEC San-ei Instruments)	19 animal corneas	Corneas undergoing larger treatments were subject to greater arises in temperature for longer periods of time - what effect does this thermal loading have on wound healing?
(Murphy et al., 2001)	To analyse the temperature change induced on the cornea by the non-contact aesthesiometer	6T62 Thermo Tracer (NEC San-ei Instruments) & non-contact corneal aesthesiometer	Unclear	Identified a threshold for detecting a cooling effect on the cornea - 0.3°C over 0.785mm^2 during 0.9 second.

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Such a temperature distribution across the ocular surface might be expected as the temperature is being differentially influenced by differing physical characteristics of the anterior eye including the following:

- An avascular cornea is surrounded by a vascularised limbal area (Fielder et al., 1981; Efron et al., 1989; Morgan et al., 1993).
- The anterior chamber is naturally shallower in the periphery compared to the centre: it has been suggested that eyes with deeper anterior chambers demonstrate less influence by body temperature on OST (Rysa and Sarvaranta, 1973, 1974).
- Steeper corneas exhibit a significantly steeper gradient across the cornea (Morgan et al., 1993): this may be because steeper corneas are more exposed and lose radiation to the environment more readily.
- The palpebral aperture accounts for the elliptical nature of the isotherms and the position of the thermal apex (Efron et al., 1989).
- The central cornea (4mm) averages 0.534mm in thickness, compared to the peripheral average 0.672mm, and the area superior to the centre is reported to be marginally thicker (10µm) (Doughty and Zaman, 2000). Morgan reported a significant decrease in OST with increasing corneal thickness (Morgan, 1994).
- Blinking causes tear fluid to accumulate initially centrally, followed by rapid dispersal to the canthi (Fatt, 1992).

The influence of these factors is examined further in Chapters 3 and 4.

No significant inter-ocular difference in OST has been reported in normal eyes (Horven, 1975; Alio and Padron, 1982a; Kocak et al., 1999). Mapstone found differences between normal eyes no greater than 0.4-0.6°C (Mapstone, 1968c). Alio and Padron (Alio and Padron, 1982a) noted some degree of asymmetry (central OST 0.5±0.06°C) in 51% of their subjects (n=96). In a study of 98 normal subjects, Morgan (Morgan et al., 1993) found no significant difference in eye temperature with respect to right vs. left eye, or first eye vs. second, and it was established that 95% of the subjects had an inter-ocular difference within 0.53°C.

1.5 Factors affecting OST

It is obvious that OST will be affected by both intrinsic and extrinsic factors. A summary of previous studies that have examined some of these influences using both contact and non-contact techniques of temperature measurement follows:

1.5.1 ENVIRONMENTAL INFLUENCES ON OST

1.5.1.1
Time of Day

There are known diurnal variations in some physical parameters of the anterior eye, so it might be expected that OST undergoes some cyclical change. For the average patient, tear film stability is low early in the morning, and tends to rise to equilibrium level between 10 am and 12 noon (Patel et al., 1988). Corneal thickness decreases rapidly on waking, and assumes a normal value after a couple of hours (Du Toit et al., 2003), although some studies have reported a cyclical variation, where a further but slower decrease occurs in the afternoon (Doughty and Zaman, 2000). Corneal sensitivity recovers upon wakening and reaches normal levels after about four hours (Du Toit et al., 2003). Other explanations for any diurnal variation in OST may be related to blood flow and aqueous humour dynamics, which are known to affect OST (Mapstone, 1968b; Wachtmeister, 1970). However, blood pressure seems to vary without real pattern [although most agree on a midday peak (Colquhoun, 1971)], and intraocular pressure increases from waking to midday, after which it falls to reach a minimum by late afternoon (Pointer, 1999). Human body temperature is at its lowest on waking and reaches a maximum late afternoon (Colquhoun, 1971).

Early published studies in ocular thermometry collected data in the afternoon but no reason was given (Schwartz, 1965; Mapstone, 1968a, 1968b, 1968c, 1968d). Schwartz reported a dependency on time of day in a study of 131 subjects, but the data was not published (Schwartz et al., 1968). Morgan investigated the diurnal variations of ocular temperature in two subjects every hour over an 11 hour period. After applying a corrective factor to allow for the ambient rise in room temperature, both subjects showed maximum temperature first thing, falling to a minimum late morning (11am) and reaching a more constant level throughout the afternoon and evening (Morgan, 1994). Applying this to his further work, he avoided the first two hours after waking to collect data. Du Toit and colleagues found in 20 subjects that corneal temperature was 1.1°C higher in the morning and seemed to return to baseline after 2pm (Du Toit et al., 1998). No other studies appear to consider time of day. This topic is examined in this thesis in Chapter 2.

1.5.1.2
Room Temperature

Given the accepted principles of thermal equilibrium, It is expected that surrounding temperatures should influence OST. Many studies have sensibly attempted to maintain stable room temperatures during experiments, but there are few studies that have investigated its direct influence.

Schwartz (using thermistor probes) noted a linear decrease in ocular surface temperature of rabbit eyes with decreasing environmental temperature: 0.23°C for 1°C rise in environmental temperatures between 2.2-27.5°C (Schwartz, 1965). Using a bolometer, Mapstone recorded the corneal temperature of 4 human subjects, seventeen times over an eight week period, and demonstrated a 0.15°C increase for 1°C rise in environmental temperature over a range of 18-27°C (Mapstone, 1968b). Kolstad demonstrated a reduction in corneal temperature (glass probe thermistor) with cold conditions (n=2) (Kolstad, 1970). Both Freeman & Fatt and Rysä & Sarvaranta observed an essentially linear decrease with ambient temperature drop, despite using very different methods (Freeman and Fatt, 1973; Rysa and Sarvaranta, 1974). Hørven demonstrated a positive correlation between room temperature and corneal temperature using thermistors on 40 subjects (Horven, 1975). More recently, Hata and colleagues observed temperature decrease in cool conditions (Hata et al., 1994). Morgan observed the change in corneal temperature in one subject over 21.7-30.4°C and found a mean rate of change of 0.21°C per degree rise in room temperature (Morgan, 1994).

1.5.1.3
Humidity

There are few studies that specifically examine the influence of humidity on OST. In a thermistor probe study, Schwartz showed a change of +1°C per 6% humidity (range 30-60%) on rabbit eyes (Schwartz, 1965). However, in a later study (Schwartz et al., 1968) no relation between radiometric readings of ocular temperature and humidity was reported. Freeman and Fatt also found humidity changes to have a minimal effect on the ocular temperature of rabbits (Freeman and Fatt, 1973).

The most likely route for influence of humidity on OST is via changes in the tear film. Korb has shown that lipid layer thickness can double if humidity increases from typical room conditions of 40-50% to 100% (Korb et al., 2002), although interestingly, it has been noted that blinking is unchanged in saunas [discussed by (Tsubota, 1998)]. Where the lipid layer is absent or defective tear evaporation is increased (Craig, 1997): dry eyes show an increased rate of tear evaporation (Craig et al., 2000; Rolando and Zierhut, 2001), and an increased rate of cooling after a blink (Morgan et al., 1995, 1996). The literature appears to support the theory that as humidity increases, lipid layer thicknesses increases, and therefore, tear film stability will increase: less cooling will occur in the tear film. However, it is unlikely that sufficiently large changes in humidity have been examined with respect to OST in order to explore these ideas.

1.5.1.4
Air Flow

Studies on the thermal conductance of skin have shown that heat transfer increases when there is surrounding air flow. Mapstone sensibly tried to exclude draughts from his experiments, but he did not specifically examine its effects (Mapstone, 1968a, 1968b, 1968c, 1968d). Freeman and Fatt positioned an air blower at various distances from a rabbit eye and measured temperature using a contact thermistor probe against the corneal surface. The cooling effect of air velocity increased with air velocity and had much greater effect at lower air temperatures. At typical room temperature (21°C), corneal temperature fell by 4 degrees when the air velocity increased from 0 to 9mph (Freeman and Fatt, 1973). Morgan used a 'thermal cubicle' for his camera set-up to exclude draughts (Morgan, 1994).

1.5.1.5
Room Adaptation

If prior to the measurement, a subject has been in a much warmer or colder environment, it is logical that this could affect the assessment of OST. In his work on anaesthetised rabbits, Schwartz ensured at least 35 minutes of room adaptation (Schwartz, 1965); Mapstone chose 15 minutes as a suitable period of adaptation for his subjects - neither gave specific reasons. The '15 minute rule' seems to have been followed in an arbitrary fashion by some groups (Alio and Padron, 1982b; Gugleta et al., 1999), and published works of others appears to have ignored it (Fatt and Chaston, 1980; Efron et al., 1988; Efron et al., 1989). Rysä and Sarvaranta found that both rabbit and human OST stabilised after 20 minutes in the cold chamber (Rysa and Sarvaranta, 1974), but an earlier study found that stabilisation varied from 20 to 45 minutes (Rysa and Sarvaranta, 1973). In his PhD thesis, Morgan examined the effect of room adaptation on 9 subjects who had previously been outside (at 6ºC). He concluded that 18 minutes should be chosen as a suitable adaptation period, despite considerable inter-subject variability. A protocol of twenty minutes was adopted for all his subsequent work (Morgan, 1994).

1.5.2 THE EFFECT OF SUBJECT VARIABLES ON OST

1.5.2.1
Effect of Age

A negative correlation between age and OST been reported (Rysa and Sarvaranta, 1973; Horven, 1975; Alio and Padron, 1982b; Girardin et al., 1999). Alio and Padron (1982) demonstrated 0.018ºC decrease in OST per year. In his 1993 study of 98 normals, Morgan found no correlation (Morgan et al., 1993), but he showed statistically a change of -0.01ºC change in OST per year increase in age (Morgan et al., 1999),

There are several features of the anterior eye related to OST that show age-related change: there is conflicting evidence about the effects of age on the tear film (Korb et al., 2002), but it is generally agreed that volume, evaporation and lipid layer thickness are constant in the normal eye with increasing age, despite changes in tear production and stability: the tear film becomes less stable with age (Patel and Farrell, 1989). The majority of published evidence suggests that there is no substantial change in central corneal thickness with age (Doughty and Zaman, 2000). Corneal topography changes with age: with the rule astigmatism changes to against the rule, particularly in men (Goto et al., 2001), which may be a factor in decreased tear film stability. The age related decrease in anterior chamber depth [0.13mm per decade from 20 to 70 years of age (Spooner, 1983)], and the increase in intraocular pressure with age (Pointer, 1999) are more likely to cause OST to increase with age according to the literature (Mapstone, 1968b; Wachtmeister, 1970).

1.5.2.2
Gender and ethnicity

One study has demonstrated significantly lower OST in women compared to men (Girardin et al., 1999), despite a higher body temperature. However, the authors admitted an age bias: the males in their study were mostly older than the female participants. Other studies have shown no correlation between OST and gender (Schwartz et al., 1968; Horven, 1975; Alio and Padron, 1982b; Morgan et al., 1993).

Relevant gender differences in the anterior eye have previously been demonstrated: women are reported to have a higher tear evaporation rate than men (Tomlinson and Giesbrecht, 1993). The relation between gender and corneal thickness has been subject of few studies and as such is seen as unimportant (Doughty and Zaman, 2000). Corneal shape does not seem gender-related, except in older age groups (Goto et al., 2001), as mentioned in Section 1.5.2.1. Corneal curvature was significantly steeper in women over 50 compared to age-matched men in a large Scandinavian study (Eysteinsson et al., 2002). Men appear to have deeper anterior chambers than women of similar age (Jansson, 1963), and females generally record a higher IOP value than age-matched males (Pointer, 1997; Eysteinsson et al., 2002).

No significant difference in OST due to race has been found (Schwartz et al., 1968; Morgan et al., 1993). Few anterior eye studies have simultaneously compared different races with regard to tear film, corneal thickness, etc. Differences in corneal thickness due to ethnic background are uncertain (Doughty and Zaman, 2000); corneal curvature of Asians and Caucasians show no significant difference (Matsuda et al., 1992).

1.5.2.3
Body temperature

A strong positive correlation between OST and body temperature (measured orally and aurally) has been demonstrated by several studies (Schwartz, 1965; Schwartz et al., 1968; Mapstone, 1968b; Freeman and Fatt, 1973; Rysa and Sarvaranta, 1974; Horven, 1975; Morgan et al., 1993; Girardin et al., 1999).

1.5.2.4
Corneal topography

Steeper corneas have been shown to have a steeper thermal gradient (Morgan et al., 1993): it has been proposed that this is due to increased evaporation from an increased surface area, or a more 'exposed' area.

1.5.2.5

Anterior chamber depth

In a cold chamber, Ryså and Sarvaranta (Rysa and Sarvaranta, 1973) noted that a fall in OST was slower in eyes with deeper anterior chambers, and this was also confirmed by comparing rabbits (larger anterior chamber) to humans (Rysa and Sarvaranta, 1974). It was suggested that the internal effect of the fall in body temperature is slower to reach the anterior eye (through the deeper anterior chamber), and therefore, a warming effect persists longer.

1.5.2.6

Corneal thickness

Du Toit and colleagues (Du Toit et al., 1998) reported a significant increase in corneal thickness upon waking, whilst both OST and sensitivity had decreased: under these circumstances corneal thickness correlated with temperature. Morgan and colleagues (Morgan et al., 1993) found no significant relationship between corneal thickness and OST, although his PhD thesis (Morgan, 1994) reports a significant ($p<0.05$) negative correlation between OST and corneal thickness.

1.5.2.7

Other ocular parameters

No significant correlations between OST and visual acuity, palpebral aperture, horizontal visible iris diameter have been established (Morgan, 1994).

1.5.3 OCULAR DISEASE AND SURGERY

It has been demonstrated that OST increases when blood flow to the anterior eye is increased, or anterior uveitis is present (Mapstone, 1968b, 1968d; Mikesell, 1978; Morgan et al., 1993). Results from studies with carotid artery stenosis subjects show that there is a significant negative correlation between ocular temperature and degree of stenosis (Mapstone, 1968b; Horven, 1975; Morgan et al., 1999). Mapstone boldly deduced that if an abnormal temperature difference between two eyes exists, it must result from differences in blood supply to the anterior segments. He found a positive correlation between maximum OST and duration of ciliary injection. Efron and colleagues (Efron et al., 1988) induced hyperaemia in the bulbar conjunctiva and found a positive correlation between OST and grade of redness (McMonnies scale), corresponding to 0.5ºC for a 3-grade change in redness. In a small, observational study, Fielder and colleagues (Fielder et al., 1981) noted that arcus begins in the warmer parts of the 'cornea' (superiorly and inferiorly), and if arcus was unilateral it was the warmer eye that was affected. It was suggested by the authors that increased capillary permeability (in response or as result of increased temperature) may explain their findings. Thermography has been used to examine the temperature effects of postherpetic neuralgia - the eye of the affected side was significantly colder, but skin temperature was not (Cardona et al., 1996; Gispets et al., 2002). Morgan explained that the increase in sympathetic innervation thought to occur with this condition was the likely mechanism of lower OST (Morgan, 1994).

There are conflicting results from the few studies that have examined the effect of posterior eye conditions on OST. Some studies showed no correlation between choroidal abnormalities and OST (Mapstone, 1968b), whilst others (Wachtmeister, 1970) show a 60% correlation, seemingly irrespective of tumour position. Gugleta et al (Gugleta et al., 1999) suggested that retrobulbar haemodynamics influence OST and is has been argued that peripheral blood flow may show parallel changes with blood flow variation in the eye (Guthauser et al., 1988). However, it is most likely that any metabolic heat produced by a choroidal tumour will be dissipated by the retina (as a vascular area of high metabolic activity), and as a result, the thermal gradient will be minimal and thus less likely to affect radiated OST.

Change in ocular temperature has been described as an indicator of the stages of wound healing and correlates well with inflammatory responses in rabbits (Coles et al., 1988). It has been demonstrated that cooling eyes post-operatively in cataract surgery reduces inflammation and improves comfort (Fujishima et al., 1994; Fujishima et al., 1995). More contemporary is the use of thermography during refractive surgery. Betney and colleagues (Betney et al., 1997) showed that OST increased during photorefractive keratectomy (PRK) surgery, to levels at which proteins can denature, but did not find that ablation depth or duration of procedure correlated with OST. This contrasts with the findings of Maldonado-Codina (Maldonado-Codina et al., 2001) who demonstrated that corneas undergoing larger treatments were subject to greater rises in OST for longer periods of time.

1.5.4 BLINKING

Many studies have taken temperature measurements in between blinks, recognising that closing the eyelids exposes the anterior eye to the warming effect of the vascular tarsal conjunctiva and fresh tears from the relatively warmer lacrimal gland and hence a rise in OST (Hill and Leighton, 1965b). An initial rise in temperature is seen straight after a blink, followed by a rapid decrease as heat is lost by convection and radiation (Efron et al., 1989). Mapstone recorded a 1.5°C rise in OST after five minutes of lid closure, and a similar decrease when blinking was prevented for long periods (Mapstone, 1968b). Several studies have demonstrated an increase in OST with eye closure (Hill and Leighton, 1965a; Mapstone, 1968a; Fatt and Chaston, 1980; Martin and Fatt, 1986).

Blinking spreads warm tears over the ocular surface, and the globe will rotate upwards to align the anterior eye to the vascular palpebral conjunctiva (Vandersteen et al., 1984). Heat transfer from the tear film to the environment occurs immediately and a decrease in OST over time is observed post-blink (Hill and Leighton, 1965b; Efron et al., 1989). The tear film destabilises after a blink which may explain the continued reduction in OST, but the exact mechanism by which tears destabilise is not fully understood. Efron demonstrated that subjects whose corneas cooled more slowly had the capacity to avoid blinking for longer periods, suggesting greater tear stability and/or increased tear film thickness (Efron et al., 1989). The stimulus to blink is still under debate: the change in OST following a blink has been suggested as a stimulus to blinking (Hata et al., 1994), and the cornea and conjunctiva have been found to be sensitive to a cooling stimulus, such as tear film thinning (Murphy et al., 1999; Murphy et al., 2001). The effect of different types of blink on OST have been examined: forceful blinking produced a greater peak temperature rise upon opening the eyelids and faster cooling, whereas flick blinking a lesser effect (Morgan, 1994). The effect of blinking on OST is examined in Chapter 4.

1.5.5 DRY EYE & ARTIFICIAL TEARS

Patients who complain of dry eyes often include the descriptive terms 'hot' or 'burning' in their symptoms (Du Toit et al., 2001). In dry eyes, the mean OST across the anterior eye appears greater, and there is a greater difference in OST over the corneal centre and the limbus, and also a faster rate of cooling post-blink (Morgan et al., 1995, 1996). Rapid cooling of the dry eye may be related to the reduced tear stability and increased evaporation (Craig et al., 2000), and blink rate (Efron et al., 1989). Dry eyes are known to be more affected by dry environmental conditions, in that their blink rate significantly increases (Nakamori et al., 1997), and corneal thickness has been shown to be reduced in dry eye (Liu and Pflugfelder, 1999; Guzey et al., 2002), thought to be a result of thinning related to increased evaporation and osmolarity in dry eye patients.

Artificial tears (of various temperatures) have been shown to lower OST and improve subjective comfort (Fujishima et al., 1997). In his thesis, Morgan showed that 1.4% polyvinyl alcohol reduced OST to a greater degree than saline. It is likely that the viscosity of the solution is important: aqueous artificial tears appear to cause an uneven thickening of the precorneal tear film, particularly superiorly (Shimmura et al., 1998). The effect of punctual occlusion on ocular temperature was shown to be inconclusive (Morgan, 1994).

The effects of artificial tear substitutes on OST are examined in Chapter 7 of this thesis.

1.5.6 CONTACT LENS WEAR

There have been few reports in the literature observing temperature of the anterior eye during contact lens wear, despite the use of terms like 'burning' or 'hotness' by symptomatic contact lens wearers, and known effects of contact lenses on anterior eye physiology.

There are short term changes in the tear film when a contact lens is inserted, but the long term changes are not clear from the literature:

- A contact lens disrupts the integrity of the lipid layer and increases tear film evaporation (Korb et al., 2002). This increase in tear evaporation has not been found to be related to the water content of a soft HEMA lens (Cedarstaff and Tomlinson, 1983). The stability of the post-lens tear film depends on factors such as blinking, lens edge profile, tear production and drainage and lens material (Lin et al., 2003). Studies have demonstrated that both hard and soft contact lens wearers blink more often than non-contact lens wearers, although a few cases exhibit significantly less blinking (Tomlinson, 1992). At best, a thin lipid layer will form over a hydrogel lens, but with a rigid lens that moves more, the lipid layer will be absent (Holly, 1981).
- A vascular response to soft contact lens wear has been demonstrated (McMonnies et al., 1982): it is logical to suspect that this will have an increased warming effect on OST.

Most of the studies on contact lenses and OST have used contact methods of temperature measurement and been observational in nature. It is difficult to compare such studies as the environmental factors and measurement techniques differ between them. Hill and Leighton demonstrated an increase in OST behind a scleral contact lens embedded with a thermistor, an effect that increased as the palpebral aperture narrowed (Hill and Leighton, 1965a, 1965b). Hamano and co-workers reported no difference in temperature of more than 0.5ºC between the ocular surface with or without a rigid contact lens in place (Hamano et al., 1969). Using a bolometer, Fatt and Chaston demonstrated three eyes wearing a hard contact lens had a temperature 0.5-1.5ºC below that of the same eye without a contact lens, but three eyes wearing a soft lens had a temperature never more than 0.5ºC below the same eye without a contact lens (Fatt and Chaston, 1980). Martin and Fatt used a contact method to show that contact lenses of higher water content caused a smaller rise in ocular surface temperature than lenses of lower water content. Their model of heat transfer predicted a small increase in anterior corneal temperature beneath a hydrogel contact lens, but the authors felt that this would be insignificant compared to environmental influences(Martin and Fatt, 1986). Most recently, Montoro et al observed an irregular thermal pattern in contact lens wearers (Montoro et al., 1991). With increased instrument sensitivity, it may be that temperature changes behind contact lenses become significant, particularly in the advent of the growing popularity of continuous wear. It is established that prolonged eye closure results in an associated sub-clinical state of inflammation, which is comparable to that during contact lens wear (Sack et al., 1997). The use of soft contact lenses for therapeutic purposes in a wide variety of disorders has also become more extensive as a result of developments in contact lens materials and the increasing popularity of refractive surgery.

The effects of contact lens wear on OST are examined in Chapter 6 of this thesis.

1.6 SUMMARY

It is apparent from the literature that the potential use of OST in monitoring ocular physiology has been well-recognised. As early as 1970, it was acknowledged that technology was the key to progress in this area (Wachtmeister, 1970), and that improvements in sensitivity and equipment cost would be necessary to make this technique a useful research or clinical tool (Efron et al., 1988). It appears generally agreed in the literature that the OST measured upon eye opening is that of the tear film, and as such OST will be continuously affected by eyelid movement, thinning and evaporation, and ocular surface properties. The latest generation of thermal cameras offer the necessary improvements in terms of portability, cost and temporal analysis, following the parallel move in medicine towards using dynamic thermography (Anbar, 1998) as a more useful diagnostic tool. The ability to measure OST in real-time with current technology offers great opportunities for progress in monitoring ocular physiology.

1.7 AIMS OF THIS THESIS

The emphasis of this thesis is on the dynamic observations of OST following a blink. Specific aims are outlined below:

- To evaluate the technique of dynamic ocular thermography
- To attempt to establish the source of measured OST - the established literature suggests it is the tear film, but this is mainly based on theory.
- To examine the temporal changes in OST following a blink in a large group of subjects, and relate the findings to ocular physiology, e.g. tear flow dynamics.
- To examine the effects of different types of hydrogel contact lenses on OST, both *in vitro* and *in vivo*.
- To examine the effect on OST of the application of artificial tear drops - both initially and over time.

CHAPTER 2

EXPERIMENTAL DESIGN

2.1 Laboratory set-up

2.1.1 Choice of camera

In medicine, thermography is used as a diagnostic tool, rather like an x-ray, where static images are recorded and studied for signs of asymmetry or abnormality, although there is a move towards using dynamic techniques (Anbar, 1994). Previous research in ocular temperature has used static thermography, but it is evident that changes in OST may be small and rapid: the tear film is a dynamic structure and is continuously being affected by eyelid movement, evaporation and ocular surface properties (Craig et al., 2000). Efron and colleagues noted that the OST was unstable over time, and suggested that even in the absence of blinking, the cornea undergoes a cyclic variation in temperature of up to 0.78°C (Efron et al., 1989). The relative changes in OST compared to baseline will be important, especially as it is apparent that the recording of accurate absolute corneal temperature is subject to many factors (Chapter 1.5), and "abnormal" temperature changes may be masked by these "normal" variations.

The literature has indicated that temporal analysis of tear film in dry eye patients might be more useful than static imaging (Morgan et al., 1995). High-speed, video-topographic measurement of tear film build-up time provides one possibility of measuring tear-film dynamics, but is currently limited to 4 images per second (Nemeth et al., 2002). To date, thermography of the tear film in dry eye has been limited to one frame per second, as resolution was found to suffer at higher frame rates (Morgan et al., 1995). The latest infrared cameras are vastly superior to previous, with improvements in image processing and frame speeds that allow images from the camera to be transferred to a PC in 'real time' – this permits temporal analysis of temperature.

The camera used for this research (NEC San-ei Thermo Tracer TH7102MX; Figure 2.1) has its origins in industrial applications, but has also been used in equine and arthritis research.

Aston University

Illustration removed for copyright restrictions

Figure 2.1 The TH7102MX thermo-camera (NEC San-ei, Japan)

The optical system of the camera focuses and transmits the radiation from the subject: a Germanium lens is used due to its high refractive index (and therefore thin), and the high reflective loss is counteracted by anti-reflection coatings. A bandpass filter is then used to select the wavelengths suitable for the detector.

The detector used in this camera is Vanadium Oxide (VOx) which is an uncooled detector: one that can operate at an elevated temperature without the need of temperature reduction to enhance its responsivity and other radiometric characteristics. The VOx acts as a microbolometer: a sensitive thermometer whose electrical resistance varies with temperature. As the VOx array detects varying degrees of thermal radiation in a scene, the material heats up, the resistance of the material changes and pixel intensities are developed from the signal differences from the original voltage applied across the detector. VOx arrays are inherently sensitive: a chopper device to amplify signals is not required. This helps to reduce the thermal background noise of the system (Pandya and Van Anda, 2004).

The performance of the detector is a function of sensitivity, resolution, and range. Sensitivity, the ability to resolve two objects of nearly equal apparent temperature, is measured by noise-equivalent temperature difference (NEdT) and minimum-resolvable temperature difference (MRTD). NEdT, measured in millikelvins, consists of the amount of IR radiation needed to produce a signal equal to the heat (noise) created by the detector itself. The lower the noise of the system, the lower the NEdT and the smaller the detectible signal. MRTD, measured in degrees centigrade, describes how well a detector distinguishes between objects at similar temperatures. For a VOx detector, the typical NETD will be 25 millikelvins for a 50-micron pixel using an f/1.0 optical system or lens, and MRTD below 0.1° (Pandya and Van Anda, 2004). The TH7102MX thermo-camera (NEC San-ei, Japan) has a MRTD of 0.08°C at 30Hz.

In any digital thermal camera, the image resolution is defined by both the optical system and by the number of pixels on the IR detector. By reducing the pitch between sensor elements to 25 μm, an image of 320 X 240 pixels can be produced (Pandya and Van Anda, 2004), such as in the TH7102MX thermo-camera.

The detector is compensated for ambient (between lens and subject) temperature and humidity conditions via manual programming. Compensation for any high or low temperature items in the background achieved by allowing the camera to equilibrate with its surroundings for 10 minutes each session before any data is collected with the lens cap removed. The lens cap (coated with blackbody paint) is then replaced and used to externally calibrate the camera before each experiment: this allows a correction factor to be calculated by the camera to correct for any reflection of environmental temperature. Using a close-up focus lens does help to reduce the potential effect of background radiation. For internal calibration, the camera has a paddle, temperature sensor and black reference for checking the uniformity of the detector array: the paddle periodically moves in front of the sensor for this purpose.

The electronic circuitry of the camera supports image processing, compensation mechanisms, the selection of sensitivity, range, and emissivity factors as well as display modes. The LCD viewfinder permits observation of the 'live' image; there are digital (14 bit) and analogue outputs for vision and imaging applications.

The physical properties of this camera are summarised below:

- It uses an uncooled focal plane array for the detector, which makes it portable (it has its own LCD colour view-finder; total weight approx 1.6Kg: battery or mains operated)
- Self calibrating: no need for black body device.
- The detector is sensitive to 8-14μm, which would seem suitable for the emission spectrum of the anterior eye (Lerman, 1980)
- It has three frame speeds (7.5/ 30/ 60Hz), as well as static facility
- Maximum temperature resolution of 0.08°C at 30Hz, 0.16°C at 60Hz
- Accuracy of ±2% (over widest range)
- Addition of a close-up lens allows close focus at 60mm, with a spatial resolution of 100μm, and field of view 34.5mm (H) x 26.0mm (V)
- Pixel size of image 320(H)x240(V)
- Colour/ monochrome facility
- Digital and video output facility from the camera permits recording of data that can be processed at a later date, if required.

2.1.2

Camera set-up

A dedicated, temperature-controlled laboratory has been used for all the studies in this thesis; room temperature and humidity were monitored by use of a digital hygro-thermometer (accuracy ± 1%, sensitivity 0.1°C, sampling rate 10 seconds), and background radiation was reduced by the use of fluorescent room lighting only (no windows). Doors were kept closed to avoid draughts. The camera was mounted onto a slit lamp base, allowing precise alignment of the desired image using the joystick (Figure 2.2). The slit lamp was mounted on a standard ophthalmic table: the chin-rest and head-rest encouraged steady positioning of the subject.

Figure 2.2
Thermo-camera mounted on slit lamp base

Initial, gross focus of a subject's eye was obtained by observing the lashes and changes in the tear film occurring with blinks, and then the distance between eye and camera lens was checked by placing a rule of length 58mm between the lens and the closed lid (over the central cornea). The thickness of the closed lid was assumed to be ≈2mm (Bron et al., 1997). The screw-lock on the slit-lamp base was used once focus was established. The camera video output was connected to a personal computer (PC), and the signal was processed by custom-designed software (Section 2.1.3).

Internal settings for the camera during experiments were as follows:

- 0.98 was chosen to represent the emissivity of the anterior eye surface, due to its similarity in thermal properties to skin (Steketee, 1973), water (Lerman, 1980), and the work of previous researchers (Mapstone, 1968a; Rysa and Sarvaranta, 1974).
- The scale sensitivity for the screen display was set at 0.5°C: this allowed a temperature range of 4°C to be viewed. NB This was not the ultimate thermal resolution of the camera - that was determined by the programming (section 2.1.4).
- Frame rate of 30Hz, allowing image processing in real time
- Image was 256 positive monochrome, providing a linear scale where whiter shades represented warmer areas.

2.1.3

Anatomical localisation

The camera's field of view is 34.5mm (horizontal) x 26.0mm (vertical) at a 60mm working distance, represented by 320x240 pixels on the computer screen: as the average distance between canthi is about 30mm, the camera's field of view covered the entire anterior eye surface in most subjects. The thermal image of the anterior eye lacks the usual anatomical detail such as pupil and iris, which would aid accurate positioning. Although the canthi and lids can be readily localised, it is difficult to identify the corneal centre, even with the magnified view on the PC monitor (resolution of 1280x1024: Figure 2.3).

Ideally, a simultaneous on-axis visible light image would assist in localisation, but a beam splitter would disrupt the thermal profile and the 60mm working distance would result in any additional camera having to be set at an oblique angle. Anatomical localisation has proved difficult in earlier studies (Efron et al., 1989; Morgan et al., 1993): it has been assumed that the centre of the cornea lies equidistant from upper and lower lids and that the coolest point represents the centre of the cornea itself. A criticism of this supposition is that the palpebral aperture shows considerable variability between subjects and can vary within subjects according to transient fatigue. The anatomical features that are most likely to remain constant are the canthi and the lower lid margin (Bron et al., 1997). Hence alignment with the camera was achieved as follows:

- For each subject, measurements were taken with a PD rule (to the nearest ½mm) - the vertical distance from pupil centre to lower lid margin, and the horizontal distance of this vertical drop to the inner canthus
- The magnification of the system was calculated by imaging a small disc of known diameter, and measuring the size of the same on the monitor. The palpebral aperture of one subject was also measured four times with a ruler and compared with the same feature on the PC monitor. The average magnification was calculated to be 5.8 (±0.2), i.e. a typical horizontal iris diameter of 12mm was represented by a distance of 69.6mm on the monitor, and the distance from canthus to canthus was represented by 150-160mm on screen.

- Using the magnification calculations, the anatomical features of the individual subject could be mapped onto the PC monitor to allow accurate alignment between the camera optical axis and the geometric centre of the cornea. These figures (or an acetate overlay constructed from them) were used for repeated measures to minimise inaccuracy in recording the thermal profile.

OST was recorded from 23 points (see section 2.1.4) across the ocular surface: the positions of these points were selected to highlight areas of interest (central, nasal limbus, temporal limbus, superior cornea, inferior cornea), and avoid measuring skin temperature. The central nine points covered an area of approximately 4mm diameter overlying the central cornea. The superior and inferior points overlaid the outer cornea 4mm from centre. The outer points covered the limbal areas, with the furthest point lying 8mm from geometric cornea centre (i.e. extending onto the conjunctiva for typical corneal diameters; Figure 2.3).

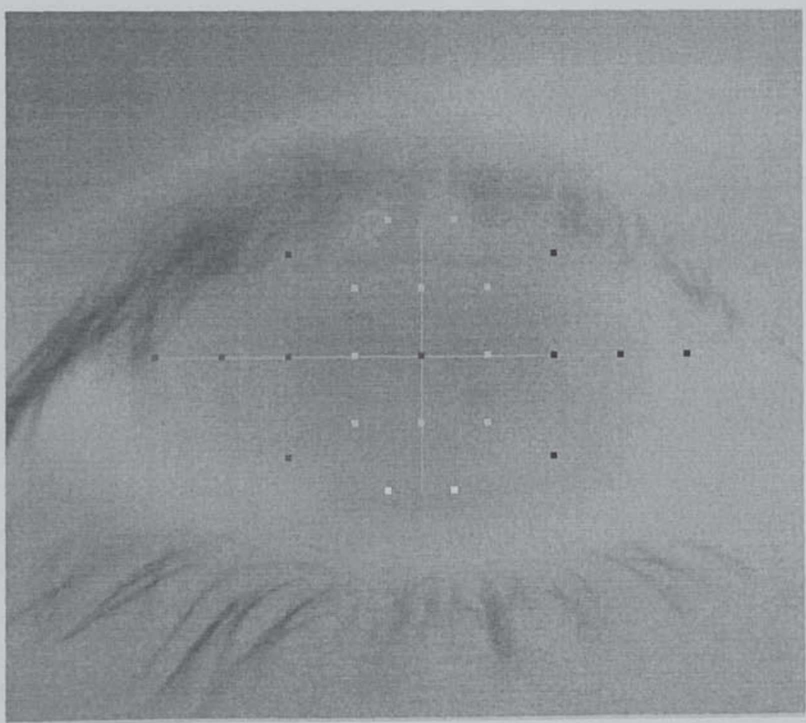


Figure 2.3
Position of the 23 OST recording points across anterior eye

2.1.4

Collection and analysis of data

Internal programming of the TH7102MX permits the positioning of 10 points or 5 boxes to continuously observe temperature at specific points, but only static images can be stored. To allow dynamic temperature information from the ocular surface to be exported and saved, purpose-written software was developed by Dr James Wolffsohn using Labview® software (National Instruments, USA).

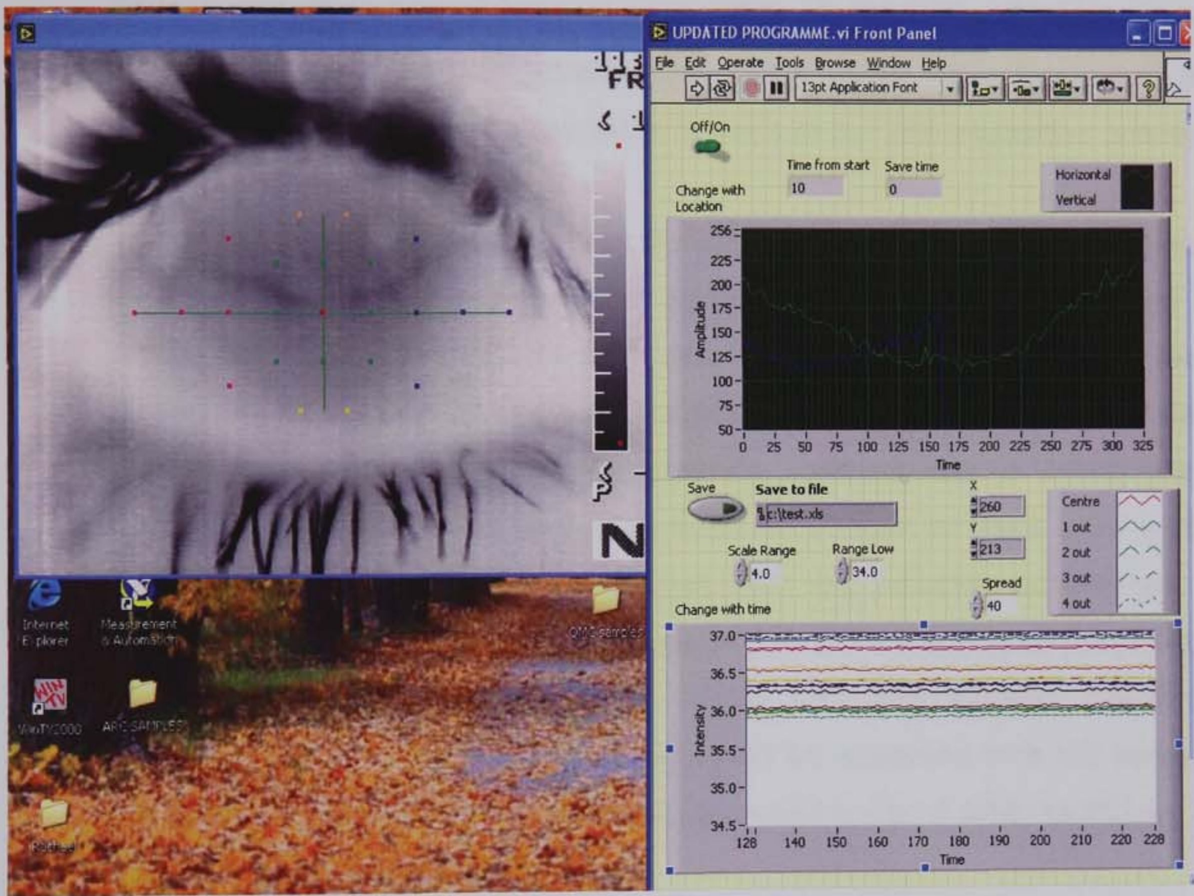


Figure 2.4
Screen view of image analysis program

To summarise the programming principles:

- To allow incremental analysis, monochrome images (8 bit) from the camera in 'run' mode are fed to a desktop PC via NTSC video output at 30Hz.
- The transferred image was the real-time image seen in the LCD viewfinder: it contained the settings for scale, emissivity, and sensitivity (Figure 2.4).

- The transferred image was the real-time image seen in the LCD viewfinder: it contained the settings for scale, emissivity, and sensitivity (Figure 2.4).
- The reference scale went from black, representing the coolest areas of the image, to white, the hottest: contained within each image, therefore, are 256 levels of luminance, or 'shades of grey'.
- The Labview® program was set up to read the temperature-generated voltage at each pixel (displayed as a luminance at that point in the image) at any desired locations, and also at the top and bottom of the scale.
- The user inputs into the temperature values indicated by the minimum (black), and the range of the scale (e.g. 4ºC), thereby directly relating pixel intensity of the image to temperature. Once these have been set, the program assigns a temperature value to each of the 256 intensities between the maximum and minimum.
- To aid selection of a suitable temperature range (and therefore, maximum sensitivity) for each subject, the software program also produced a continuous display of pixel intensities across the horizontal and vertical axes of the ocular surface (0-256). A subject can be observed prior to data collection to see whether the temperature changes are all taking place *within* 256 values: i.e. that the image does not contain all black or all white. The camera range and sensitivity can then be adjusted accordingly to ensure maximum sensitivity without loss of data from a ceiling or floor effect.
- Typically, OST variations during a typical recording session (pre-blink, during and post-blink for 8+ seconds) could be observed with the camera set for a range of 4°C, spread over 256 values, i.e. a total sensitivity of 0.016°C.
- OST was continuously recorded, along with time, in this way from 23 points across the anterior eye (Figure 2.2). The results fed directly into Microsoft Excel® spreadsheets.

Analysing the data:

Continuous recording of OST for 10-12 seconds of recording this would typically produce approximately 400 rows x 24 columns (9600 temperature measurements) in Excel. This large amount of data was managed by grouping the points into five areas: central (9 points), upper (2 points), lower (2 points), nasal (5 points) and temporal (5 points). The locations and dimensions of these areas are explained in section 2.1.3.

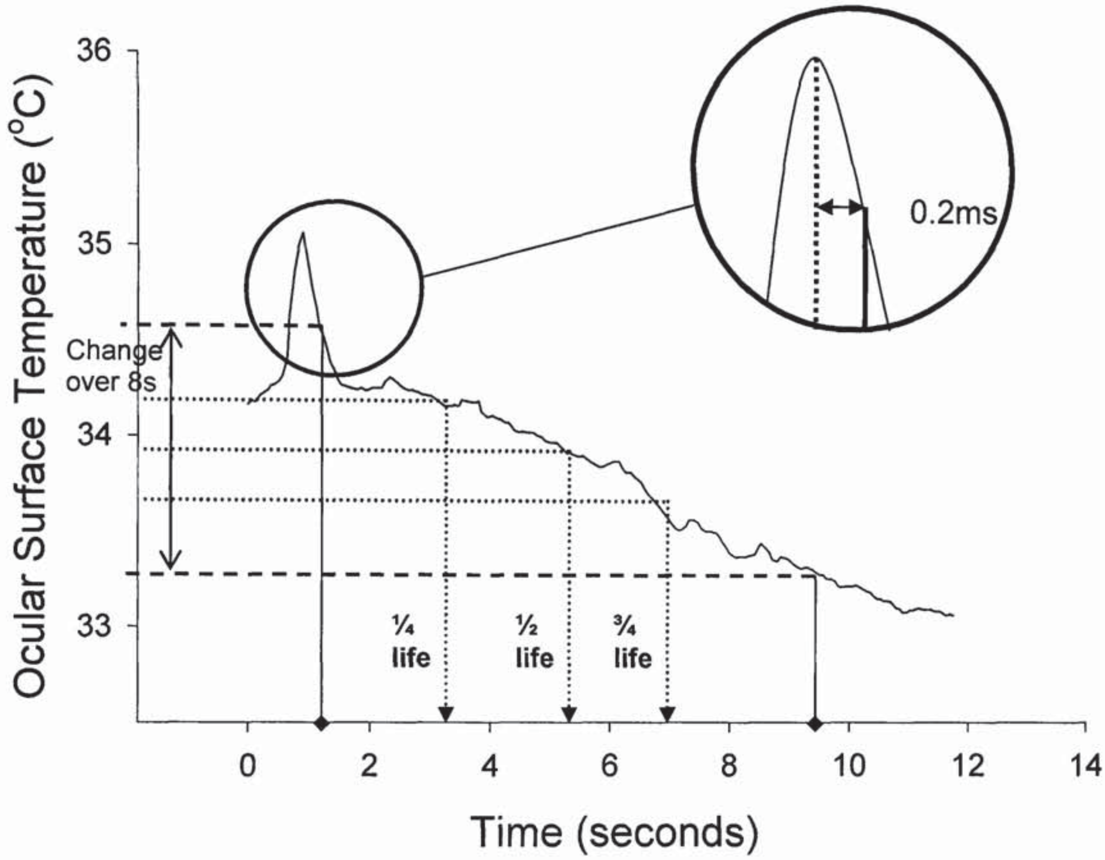


Figure 2.5
Typical temporal change in OST with blinking, showing reference points selected for analysis

Excel spreadsheet templates were used to smooth the data for each area (average of temperature within ±0.3s), and a line plot drawn to represent the change in OST over time, including pre- and post- blink (Figure 2.5).

For the recording in each area, the time corresponding to the peak (as the relatively hot eyelid covers the camera's field of view) was manually selected: the point of eye opening could be identified and initial OST post-blink could be recorded. Point of eye opening was chosen as 0.2s after the peak value (Figure 2.4). The upper lid accelerates rapidly as it descends, and slows significantly as it rises: the total duration of lid motion has been shown to be between 300-400ms (Doane, 1980; Vandersteen et al., 1984; Chauhan and Radke, 2001). This suggests that at approximately 0.15s the central OST can be recorded, and it is likely that the superior cornea becomes exposed at 0.2s. Owens and Phillips chose 40ms (0.04s) after passage of the lid to start video analysis of tear stabilization, although it is unclear whether that meant the eyelid was fully open or not: due to the dynamic nature of the tear film data may have been lost (Owens and Phillips, 2001). Once this eye-opening point was identified, the data was manipulated within the Excel template to reveal the total change in OST over eight seconds post-blink.

During data collection subjects were asked to blink normally whilst the camera was aligned and following a natural final blink, to hold their eye open for eight seconds without blinking. Previous work has identified that a forced blink results in a slightly higher initial temperature, although the rate of change appears similar (Morgan, 1994). Eight seconds was chosen as a suitable time to assess the tear film without causing reflex tearing - average blink rate under concentration has been reported as every 12 seconds (Tsubota and Nakamori, 1995). A simple rate of change for OST could be calculated using this data. However, the change in OST during the 8s post-blink period was unlikely to be linear, so the actual times taken for ¼, ½ and ¾ of such change to occur were calculated in order to fully describe the dynamic profile (Figure 2.5).

To summarise, the chosen method of analysis produced measures (for each area) of:

- Initial OST after a blink
- Change in OST for 8s after a blink
- The time taken to reach:
    - ½ of the total 8 second temperature change (half-life)
    - ¼ of the total 8s temperature change
    - ¾ of the total 8s temperature change

2.2 Evaluation of Technique

With any measurement technique, it is important to minimise variability so that smaller effects can be identified. The procedure for anatomic localisation has already been described (Section 2.1.3). The following studies examined the tolerance of the technique to changes in environment (room temperature and time of day), and patient variables (stability of head and the effect of eye rubbing). The results led to a technical protocol for using the camera, from which the repeatability of the measurements could be assessed.

2.2.1 Effect of image defocus

It was anticipated that any movement towards or away from the camera would cause defocus in the thermal image, and an apparent temperature change. It was necessary to determine the tolerance of the thermal profile to changes in camera-to-eye distance to establish whether a head restraint (such as chin rest, Velcro head strap or bite-bar) was necessary to minimise this source of inaccuracy.

2.2.1.1 Method

A model eye was constructed using an array of six light emitting diodes (LEDs) set into a resin convex dome. The LEDs were connected to a transformer, and a voltage applied to give a temperature similar to that of the anterior eye, but without the dynamic influence of the tear film. The model eye was aligned with the optical axis of the thermal camera (mounted on an optical bench) and the thermal profile of the central cornea measured with the model eye-to-camera distance altered in 1mm steps on an optic bench over a range of 45 to 75mm (Figure 2.6). Pearson's correlation coefficient was used to measure the relationship between camera-model eye separation and recorded temperature.

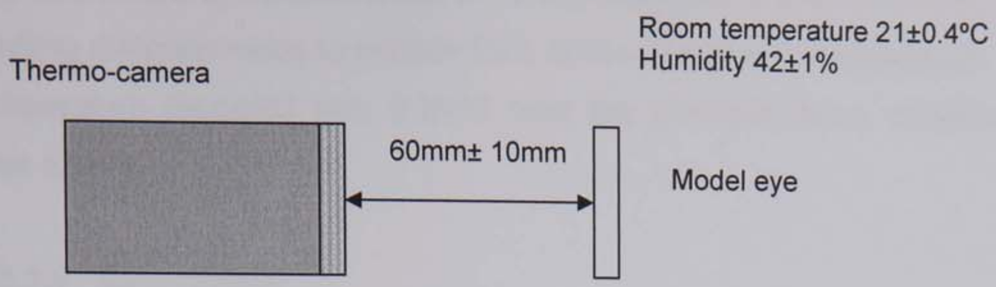


Figure 2.6
Experimental set-up to study effects of defocus

2.2.1.2 Results

A significant negative correlation was found between the central radiated temperature of the model eye and the model eye-camera separation examined ($r=-0.73$, $p<0.0005$), i.e. increasing eye-to-camera separation resulted in lower recorded temperatures (Figure 2.7).

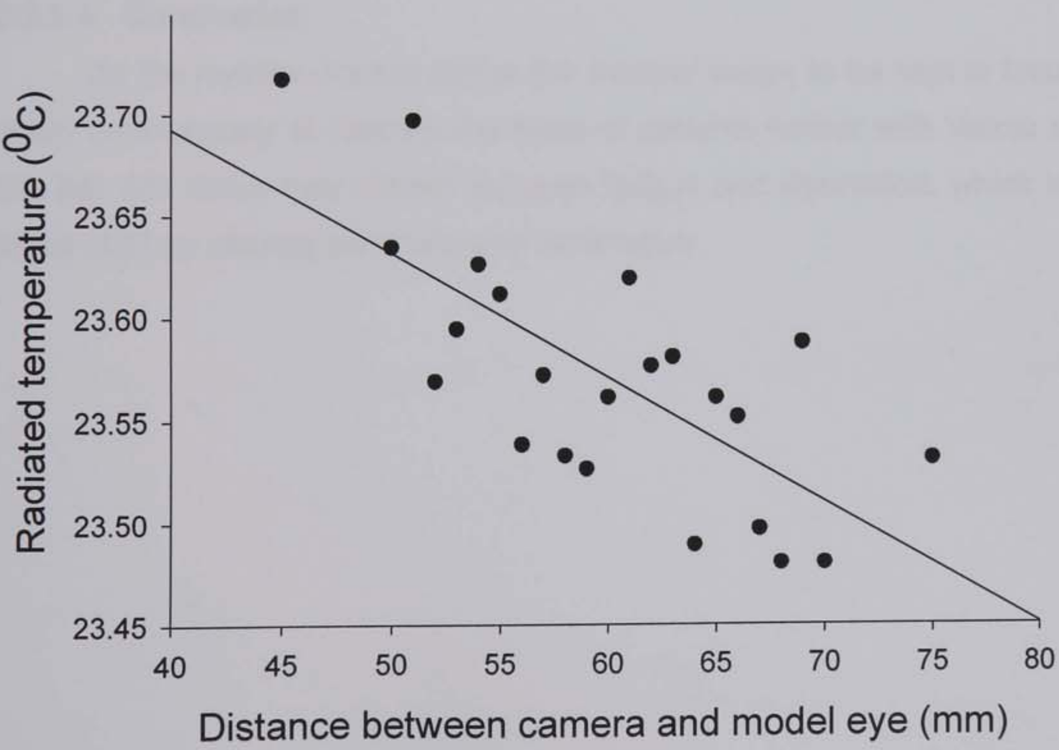


Figure 2.7
Relationship between radiated temperature and camera-eye separation

The coefficient of determination (r^2=0.53) indicated a shared variance of 53%, i.e. working distance helps to explain 53% of the variance in temperature. The range of temperature recorded was 0.24°C over the principal focus ±15mm, and 0.13°C over ±5mm.

2.2.1.3 Discussion

Increasing the separation between a radiation source and a detector will result in an apparent loss of radiation and an increased level of camera defocus. Therefore, it was expected that apparent temperature would decrease with increasing separation from the camera. The observed changes in temperature with varying eye-camera separation are small: over a range of ±5mm (as might be expected from patient stability when resting against a forehead and chin-rest), the apparent change in temperature was approximately 0.1°C; the results suggest that movement of the subject away from the camera has insignificant effect in contrast to moving closer.

2.2.1.4 Conclusion

As the joystick control allows the thermal image to be kept in focus, it would seem unnecessary to restrain the head of patients further with Velcro straps or a bite-bar and these may indeed increase fatigue and discomfort, which in turn may affect OST by altering blink rate and lacrimation.

2.2.2
Effect of Room Adaptation

If prior to the measurement, a subject has been in a much warmer or colder environment, it is logical that this could affect the assessment of ocular temperature. In his work on anaesthetised rabbits, Schwartz ensured at least 35 minutes of room adaptation (Schwartz, 1965). Mapstone chose 15 minutes as a suitable period of adaptation for his subjects: neither study gave specific reasons. The '15 minute rule' seems to have been followed in an arbitrary fashion by some groups (Alio and Padron, 1982b; Gugleta et al., 1999), whilst others have not mentioned this possible influence (Fatt and Chaston, 1980; Efron et al., 1988; Efron et al., 1989). In one study, Rysä and Sarvaranta found that both rabbit and human corneas (n=12) stabilised after 20 minutes in the cold chamber (Rysa and Sarvaranta, 1974), but in an earlier study (n=20) they showed that stabilisation varied between human subjects from 20 to 45 minutes (Rysa and Sarvaranta, 1973). In his PhD thesis, Morgan examined the effect of room adaptation on static temperature measures of 9 subjects who had previously been outside (at approximately 6°C). He concluded that 18 minutes should be chosen as a suitable adaptation period, despite considerable inter-subject variability with a protocol of twenty minutes adaptation time before thermal measured adopted for all his subsequent work (Morgan, 1994).

This study examined the adaptation period necessary for OST to stabilise in the laboratory.

2.2.2.1 Method

Twelve subjects took part in the study (6M, 6F; age 27.7±3.9yrs). The camera and laboratory set-up was as described in Chapter 2. Each subject entered the laboratory (maintained at a temperature of 22.1±0.4°C) having been elsewhere in the same building for at least 20 minutes (average building temperature approximately 19±3°C). Ocular surface temperature (OST) for the right eye was recorded (using the method described in section 2.1.4) every minute for fifteen minutes. Temperature data was collected and analysed as described in section 2.1.4. Pearson's correlation coefficient was used to compare average change in central area OST over fifteen minutes and repeated measures analysis of variance (ANOVA) was used to compare group mean values for significant change over time.

2.2.2.2 Results

A strong positive correlation between adaptation time and mean OST for the central area was observed ($r=0.95$, $n=12$, $p<0.0005$; figure 2.8).

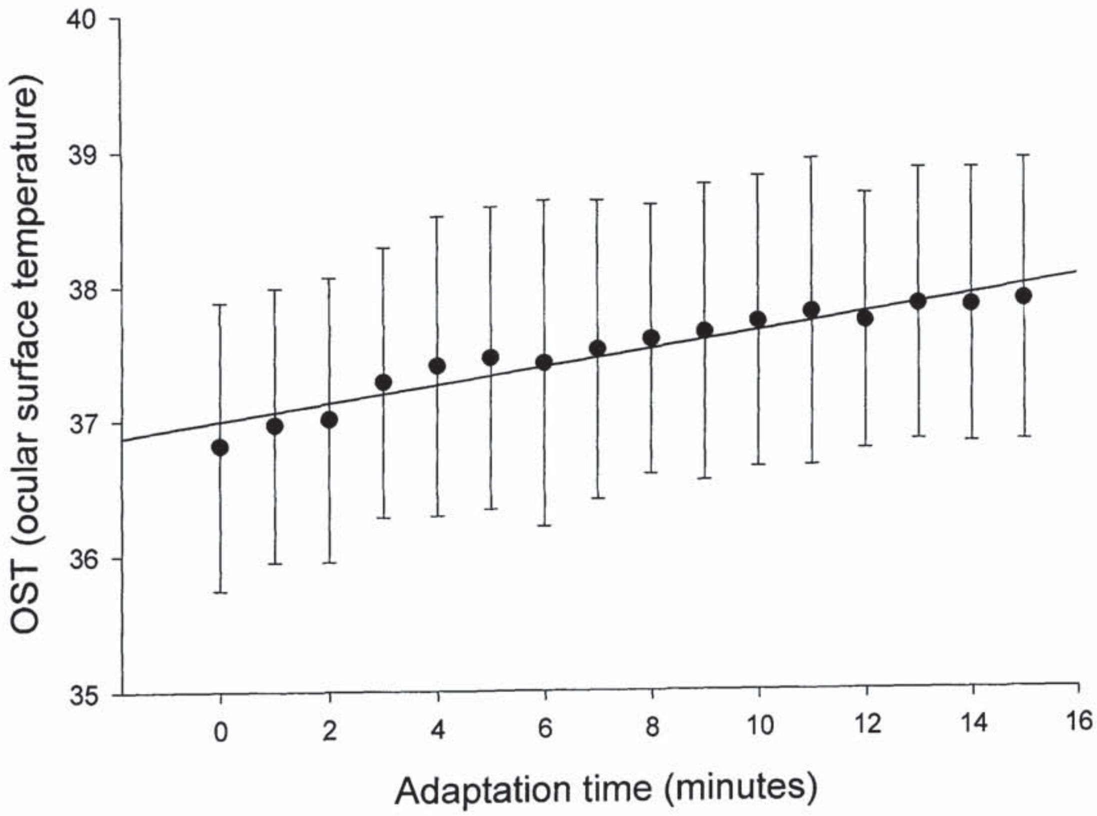


Figure 2.8
Correlation between central OST and adaptation time (error bars ±1SD)

The average difference in OST compared to baseline measures at fifteen minutes was 1.1±0.7°C (n=12). Figure 2.9 shows the average change compared to baseline at each minute for fifteen minutes (typical SD 0.6°C).

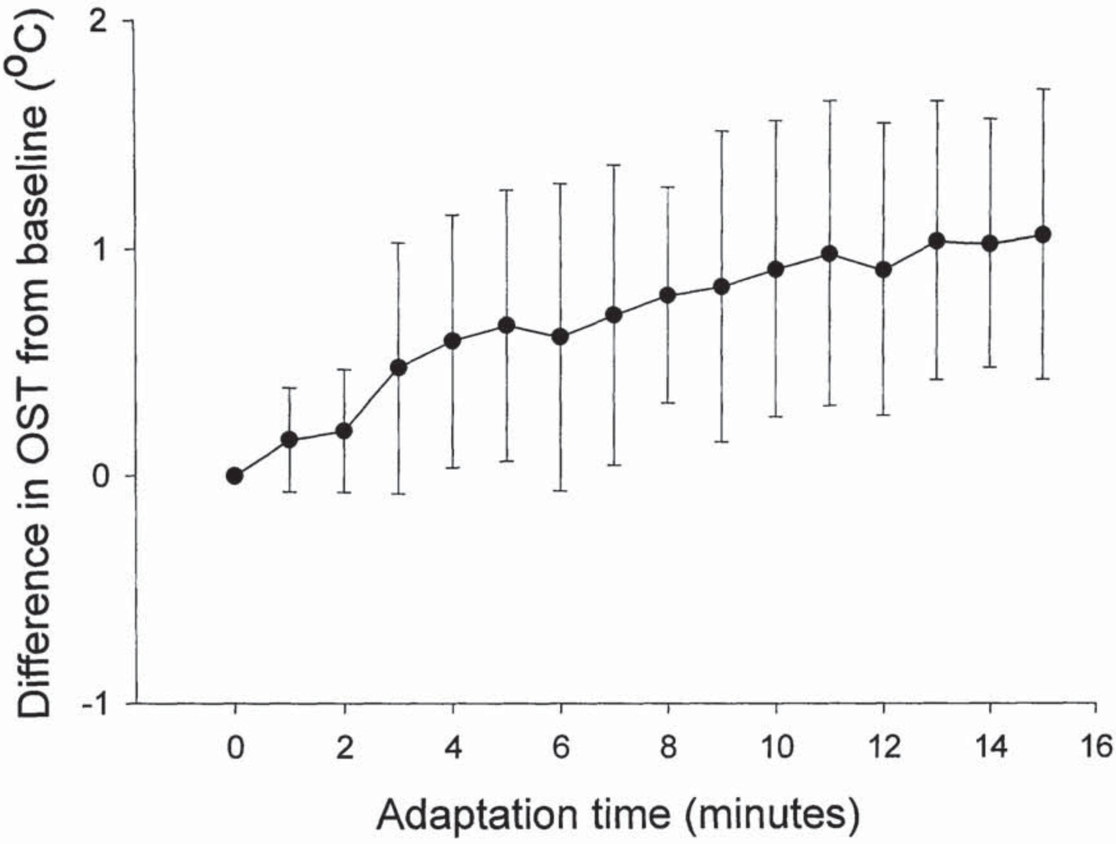


Figure 2.9
Change in central OST compared to baseline, over 15 minutes.

Repeated measures ANOVA showed that time is a significant factor overall ($F=12.40$; $p<0.0001$), and post hoc multiple comparisons analysis (Bonferroni) revealed that significant differences exist at 0,1, 2, 3, 4, 5 and 6mins, but after 6 minutes mean OST showed no further significant difference. However, graphically the results suggested some stabilisation occurs after 10 minutes.

2.2.2.3 Discussion

The results suggested average OST (n=12) increased for 15 minutes after entering the laboratory, but beyond 6 minutes, OST was not statistically significant different. This rise in temperature was perhaps expected (Rysa and Sarvaranta, 1973; Morgan, 1994), but the reasons why are likely to be a combination of the following:

- The anterior eye is kept cooler when subjects are mobile subject to increased air flow (Freeman and Fatt, 1973), and becoming stationary in the laboratory may encourage stabilisation.
- Body temperature changes with metabolic rate: when at rest a slowing metabolism will cause a decrease in body temperature (Colquhoun, 1971).
- Adapting to laboratory conditions

2.2.2.4 Conclusion

A period of time is necessary to allow OST to stabilise to the environment of the laboratory. The statistical results suggested a protocol of minimum 6 minutes adaptation for subjects in the laboratory, but the graphical plot of suggested that 10 minutes was more appropriate. The additional requirement was that subjects had been in the building for at least 20 minutes prior to this.

2.2.3
Repeatability of temperature measurement

There appears to be no reference to repeatability or reproducibility of the measurement of OST in the published literature. Repeatable OST measurements are essential if the findings are to be meaningful and also to allow consistency in repeated-measure designs. Therefore, an experiment was designed in two parts:
Part A - to examine the repeatability of successive OST measurement: within-visit repeatability is defined as the acquisition of test measurements obtained by the same operator using the same method, equipment and subjects, within short time intervals.
Part B - to examine the reproducibility of such measurements at separate sessions: the between-visit reproducibility is defined as the acquisition of independent test measurements obtained by the same operator, using the same method, equipment and subjects, on different occasions

(with reference to British Standards Institution *Accuracy (trueness and precision) of measurement methods: basic methods for the determination of repeatability and reproducibility of a standard measurement method.* BS ISO 5725 Part 2. London: British Standards Institute, 1994)

2.2.3.1
Part A - Within-visit Repeatability
2.2.3.1.1 Method

OST was recorded (technique as described in section 2.1) in four healthy subjects (2M, 2F; average age 28.25± 2.6yrs), after at least 10 minutes adaptation to the laboratory environment. Laboratory temperature was maintained at 21.1±0.4°C, humidity at 39±1%. Ten consecutive recordings were taken with the subject encouraged to blink normally (to recover from any reflex tearing effect of the 'forced stare') between each recording. Typically the separation between recordings was approximately 10 seconds, resulting in an analysis period of 200s. For each subject the average of the ten readings was calculated for initial OST after a blink and the dynamic changes in OST after a blink. Squared deviations from the mean values were compared using a one-way repeated measures analysis of variance (after Bland and Altman, 1986).

2.2.3.1.2 Results

Each subject demonstrated some degree of variation in initial OST with repeated measure (Figure 2.10).

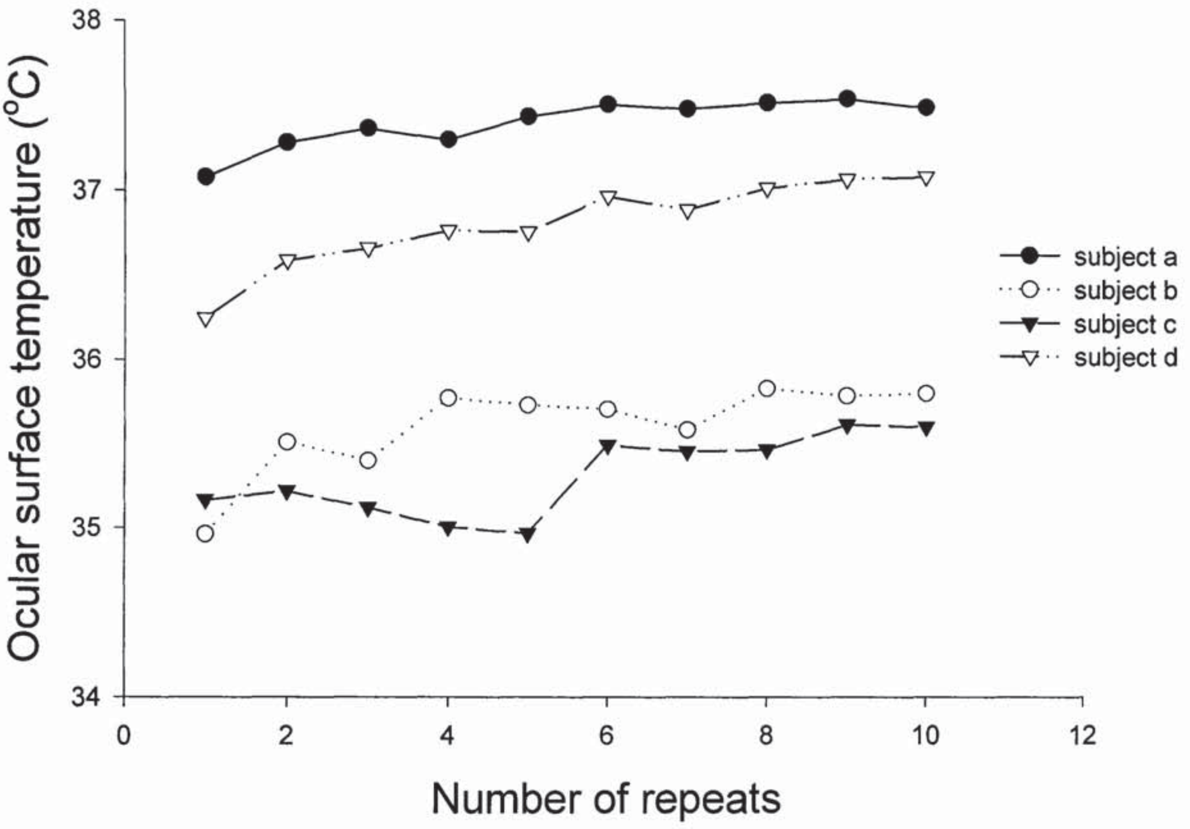


Figure 2.10
Variation in central OST over 10 repeats (n=4)

The square of the deviations from the mean values for each subject are shown in Table 2.1. The deviations around the first measures are significantly different to the others (one-way repeated measures ANOVA F=2.44, n=4, $p<0.05$; Fisher's PLSD $p<0.05$ for all pairwise comparisons).

	Squared difference between average OST (for ten repeats) and OST at each measure									
Subject	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
a	0.12	0.02	0.00	0.01	0.00	0.01	0.01	0.02	0.03	0.01
b	0.43	0.01	0.05	0.03	0.01	0.01	0.00	0.05	0.03	0.04
c	0.02	0.01	0.04	0.09	0.12	0.03	0.02	0.03	0.10	0.09
d	0.33	0.05	0.02	0.00	0.00	0.03	0.01	0.05	0.08	0.09
average	0.22	0.02	0.03	0.03	0.03	0.02	0.01	0.04	0.06	0.06
SD	0.19	0.02	0.02	0.04	0.06	0.01	0.01	0.02	0.03	0.04

Table 2.1
Differences between average of 10 measures and each measure of OST (n=4)

Since it is conventional to average a finite number of consecutive measures for analysis, the effects of cumulative averaging for each subject were calculated. Figure 2.11 displays the variation in OST with repeat, where point 1 is the OST at repeat 1, point 2 is the average OST of repeats 1 & 2, point 3 is the average OST of repeats 1, 2 & 3, and so on.

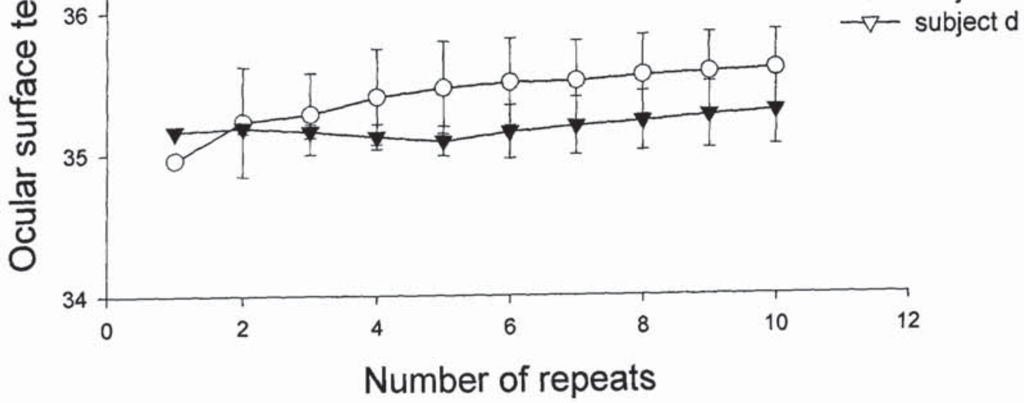


Figure 2.11
Variation in central OST (cumulative average) with repeated measure.

The plot suggests that averaging more than 3 or 4 repeats is similar to averaging more (up to ten). Therefore, the average of the first three repeats was compared to the successive measures of OST to assess repeatability: no significant difference was found (one-way repeated measures ANOVA F=1.93, n=4, p=0.12).

The magnitude of post-blink cooling was calculated for each repeat was calculated (Figure 2.12). The deviations around the mean for each subject were compared within a one-way, repeated measure ANOVA: no significant differences were found (F=1.21, p=0.38). Interpretation of the graphical plot suggested that comparing the average of the first three to successive measures was more appropriate from the graphical plot: there was still no significant difference (F=0.98, n=4, p=0.47).

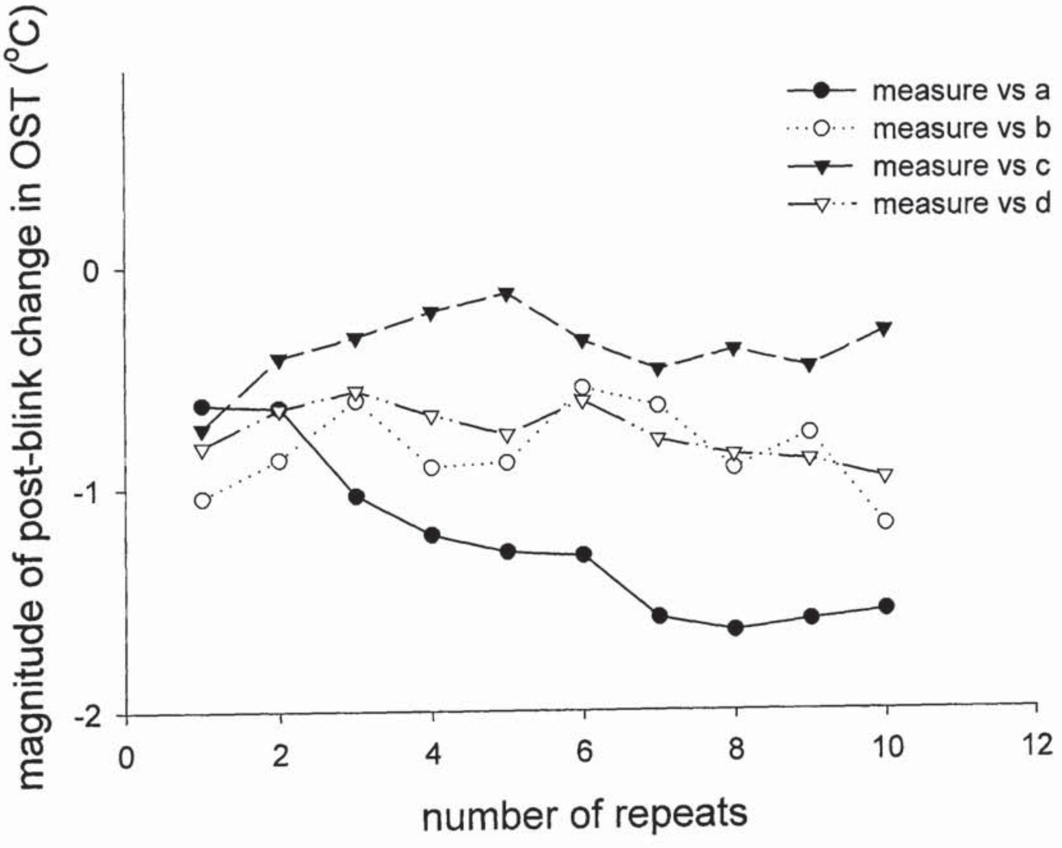


Figure 2.12
Change in the magnitude of post-blink cooling upon repeated measure, for 4 subjects

Similarly the rate of post-blink cooling was compared over repeated measure, using the values for ½ lives, i.e. the time taken (seconds) for ½ the amount of post-blink cooling to occur (Figure 2.13).

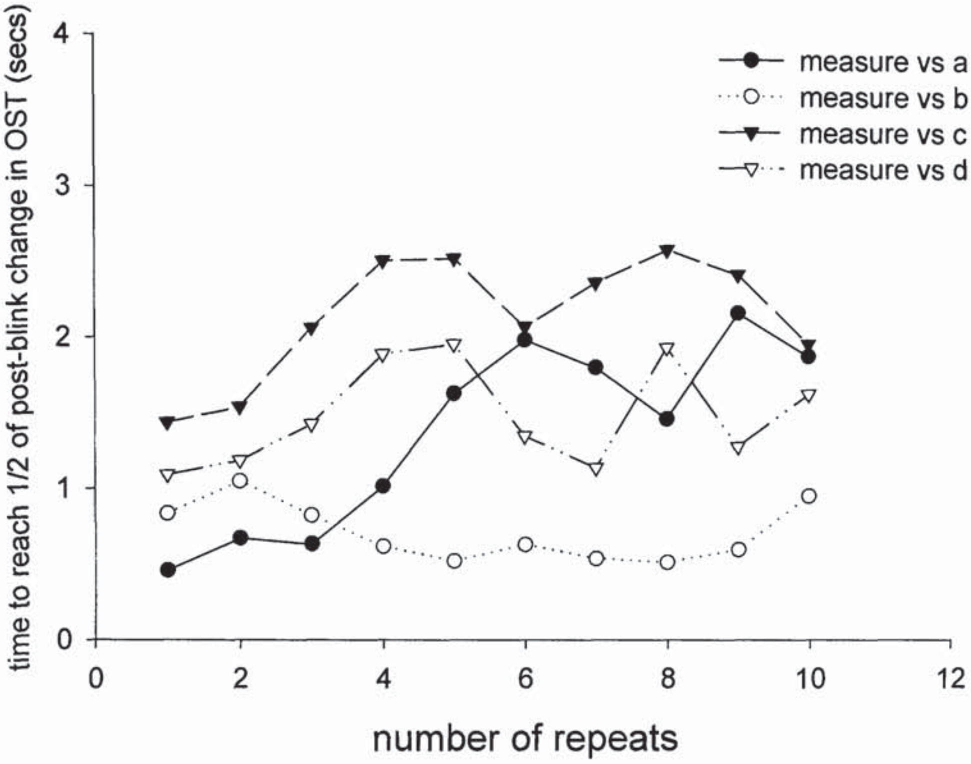


Figure 2.13
Variation in time taken to reach ½ the amount of post-blink cooling upon repeated measure, for four subjects

The deviations around the mean of ten repeats showed no significant difference between them (F=1.48, n=4, p=0.20). As expected, comparing the average of the first three to the successive measures also showed no significant difference (F=0.75, n=4, p=0.63).

2.2.3.1.3 Discussion

The results show that variation with repeated measurements of OST at a single session is initially large, but repeatability becomes acceptable if at least the first three successive measures are averaged.

2.2.3.2

Part B - Between-visit Repeatability

2.2.3.2.1 Method

Fourteen different subjects were recruited for this experiment (5M, 9F; average age 24.8±3.8yrs). The OST of each subject was recorded (as described in section 2.1) weekly over a five week period, at the same time of day, and with laboratory conditions kept constant (temperature: 20.3±1.0°C; humidity: 39±2%). Each subject was adapted to the laboratory conditions for 10 minutes prior to recording, and the average of three measures was taken.

2.2.3.2.2 Results

Most subjects displayed considerable variability in central OST (Figure 2.14) over the five week period: standard deviations ranged from 0.1 to 2.1°C. One-way repeated measures ANOVA exposed significant differences between recordings ($F=3.00$, $n=14$, $p<0.05$): post-hoc analysis (Tukey-Kramer) showed that the most significant variation occurred between weeks 1 and 2.

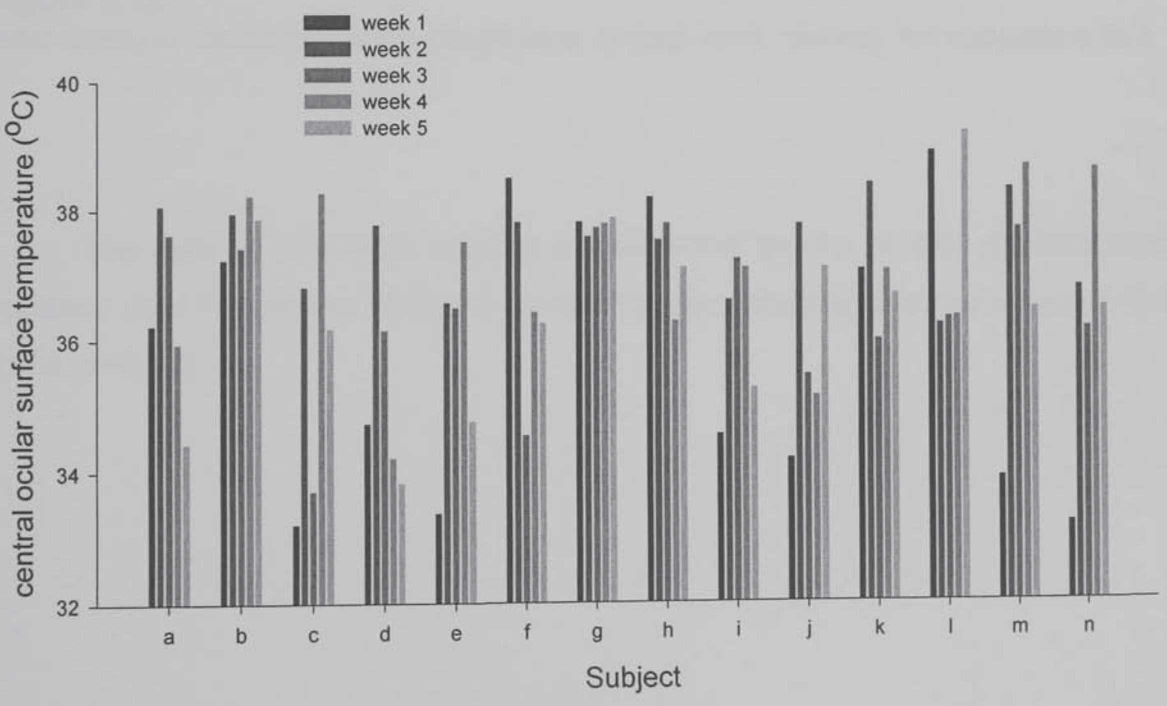


Figure 2.14
Inter-session variability in central OST for subjects a to n

The magnitude of the post-blink cooling showed less variation over five weeks: this was statistically insignificant (ANOVA comparing deviations around the mean: F=0.87, n=14, p=0.49; Figure 2.15).

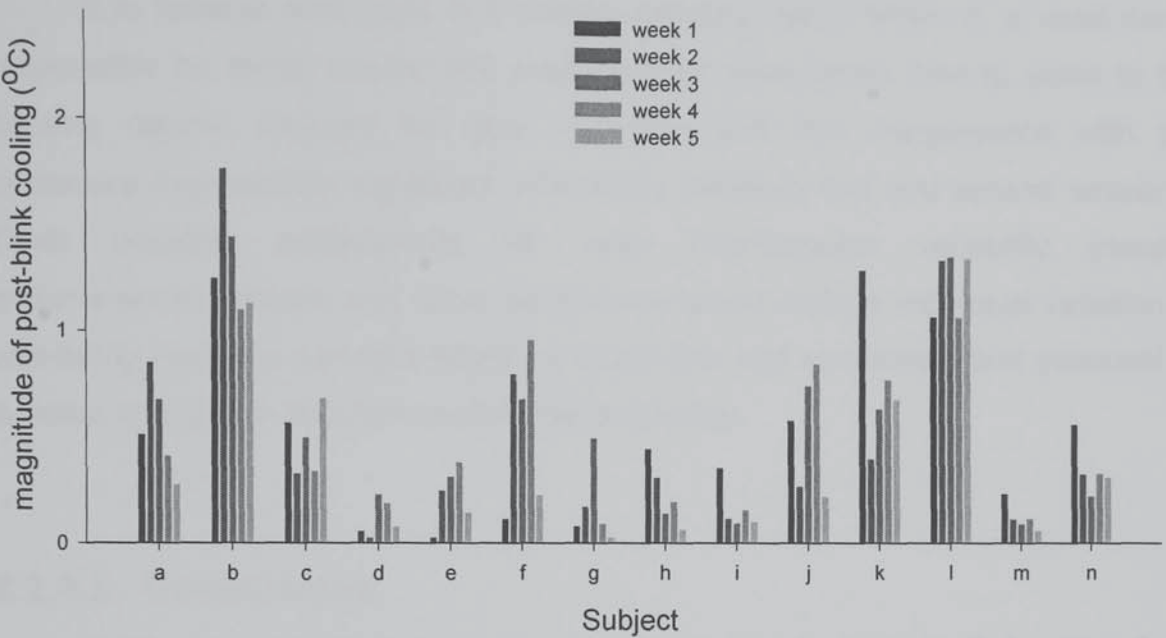


Figure 2.15
Inter-session variability in the magnitude of post-blink cooling, for subjects a to n

The rate of post-blink cooling (represented by the ½ life) showed similar variation over five weeks (ANOVA comparing deviations around the mean: F=0.88, n=14, p=0.48).

2.2.3.2.3 Discussion

The reproducibility between sessions for individuals is poor, with significant differences evident, especially between early sessions. Although lack of statistical significance suggests that the magnitude and rate of cooling is more reproducible than initial OST, standard deviations are still relatively large for theses measures.

It is feasible that there is a subject learning effect which is at least partly responsible for these results: the subjects may have taken time to settle to the blinking regime required for data collection and this inexperience with the procedure may explain significant differences between first and second sessions. Other possible explanations for wide inter-session variability (despite environmental controls and room adaptation) would include individual variation in well-being having a transient effect on blood flow and circulation, and seasonal or isolated changes in tear film quality and/or quantity.

2.2.3.3 Conclusions

OST measurements for a single subject are repeatable if the first three readings are averaged.

Observations of OST for an individual on separate occasions are less reproducible, but this might be improved with training and/or rigorous subject selection. If repeated measures of the same subjects for different conditions are required, then a new 'baseline' is necessary at each visit for reference.

2.2.4

Effect of time of day

There is evidence in the literature of diurnal variations in parameters that might affect OST.

- Human body temperature is at its lowest on waking and reaches a maximum late afternoon (Colquhoun, 1971)
- For the average patient, tear film stability is poorest in the early morning, and tends to improve to an equilibrium level between 10 am and 12 noon (Patel *et al.* 1988).
- Corneal thickness decreases rapidly on waking, and assumes a stable value after a couple of hours, although some studies have reported a cyclical variation, where a further but slower decrease occurs in the afternoon (Doughty *et al.* 2000).
- It is thought that blood flow to the eye and aqueous humour dynamics should affect ocular temperature (Mapstone 1968b; Wachtmeister 1970): intraocular pressure increases from waking to midday, after which it falls to reach a minimum by late afternoon (Pointer 1999).

Early studies in ocular thermometry collected data in the afternoon, but no rationale for this protocol was stated (Schwartz 1965; Mapstone 1968a, 1968b, 1968c, 1968d). Schwartz (Schwartz *et al.* 1968) reported a dependency on time of day in a study of 131 subjects, but the results were not presented. Morgan (Morgan 1994) investigated the diurnal variations of OST in two subjects every hour over an 11 hour period. After applying a corrective factor to allow for the ambient rise in room temperature, both subjects showed a maximum temperature when it was first measured in the morning, falling to a minimum late morning (11am) and reaching a more constant level throughout the afternoon and evening. Applying this to his further work, Morgan avoided the first two hours after waking, to collect data. In 1998, Du Toit and colleagues (Du Toit *et al.* 1998) found OST was 1.1°C higher in the morning and seemed to return to baseline after 2pm (n=20).

This study was designed to determine any diurnal variation in dynamic OST.

2.2.4.1 Method

The OST of 18 subjects (average age 28±6.3yrs; gender 9 M, 9F; right eyes only) was recorded at three different times of day: 10am, 1pm and 4pm. The procedure at each visit was as described in section 2.1, with 10 minutes adaptation prior to 3 repeated measures at each visit (section 2.2.2 and 2.2.3). Results were averaged and group means were compared using one-way repeated measures ANOVA with post-hoc comparisons.

2.2.4.2 Results

The OST for all subjects throughout the day is shown in Figure 2.16: OST increased throughout the day in twelve of the eighteen (66.7%) subjects.

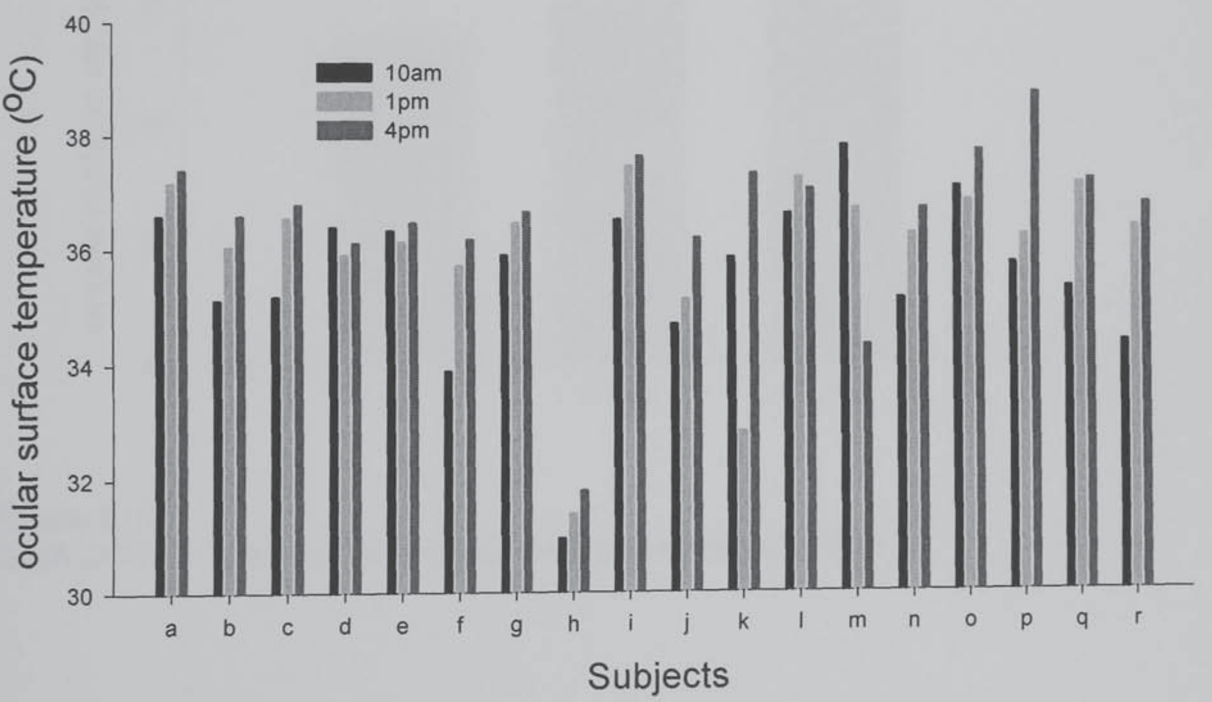


Figure 2.16
Variation in OST with time of day (n=18)

Mean values for OST are shown in Figure 2.17: the average increase in OST from 10am until 4pm was +1.4±0.9°C. Analysis of variance (repeated measures) revealed significant differences in OST between different times of day ($F=19.10$, $p<0.0005$), and post hoc analysis (both Bonferroni, Scheffe) showed the significant difference occurred between the morning compared to the afternoon recordings ($p<0.0005$), rather than morning compared to midday $p=0.36$), and midday compared to afternoon ($p=0.41$).

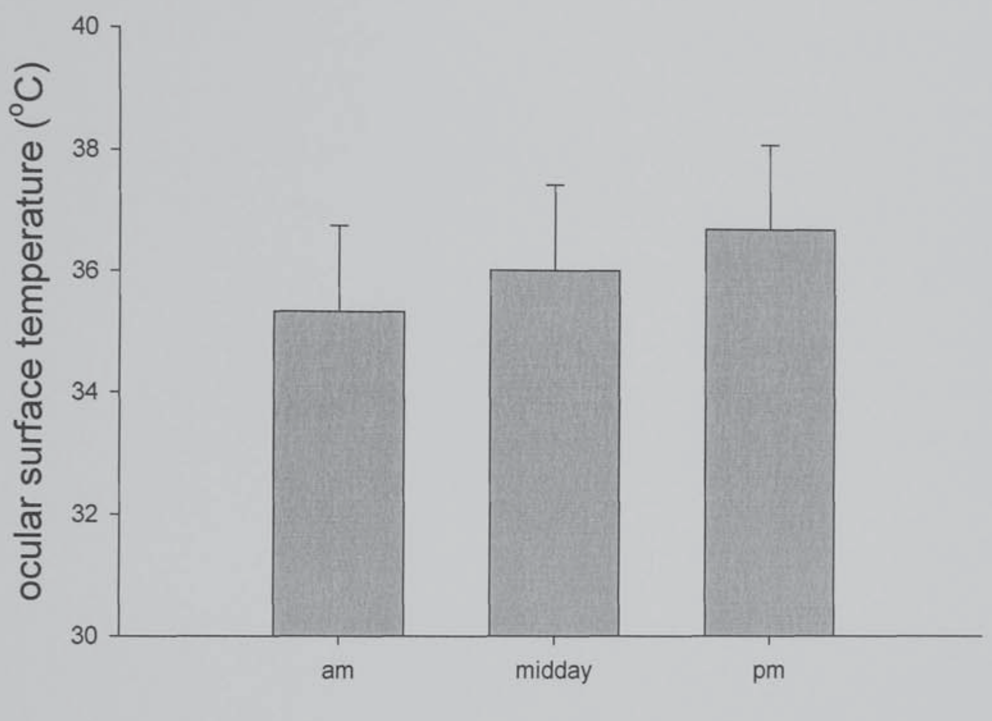


Figure 2.17
Mean OST for different times of day (n=18; error bars = 1SD)

2.2.4.3 Discussion

The results suggest that time of day is an important variable in the measurement of OST, and it would be sensible to examine subjects at similar times if values are to be compared. It appears that if measures are taken *within* a morning or afternoon session then any variation will be insignificant.

2.2.4.4 Conclusion

OST increases throughout the day for nearly all subjects, and is most likely to be highest in the afternoon.

2.2.5

Effect of eye rubbing/ contact

A study was conducted to examine whether the rationale of instructing subjects to refrain from rubbing or contacting their eyes during experiments was valid. It was felt an increase in OST might result from such action.

2.2.5.1 Method

Part 1: Effect of vigorous rubbing

Three healthy volunteers (3F age 27.7±3.8yrs) had the OST of their right eye measured using the technique described in section 2.1, during a single laboratory session. All subjects had room adapted for at least 10 minutes prior to measurement. Three readings were taken as a preliminary measure; subjects were then asked to deliberately rub their closed eye three times with the knuckles of their right index finger, and then three more recordings of OST were taken. The main features of pre- and post-rub OST were calculated using the methods described in section 2.1.3, and Student's paired t-test was used to analyse any differences found.

Part 2: Effect of eye contact

Three healthy experienced soft contact lens wearing volunteers (2F, 1M, 25.3±0.6yrs; worn hydrogel lenses for >1yr) had the OST of their right eye measured using the technique described in Section 2.1, during a single laboratory session. None had worn lenses for at least 24 hours. All subjects had room adapted for at least 10 minutes prior to measurement. Three readings were taken as a preliminary measure; then subjects were asked to make the same pincer-like action to contact their eye as if they were removing a hydrogel contact lens. The main features of pre- and post-contact OST were calculated using the methods described in section 2.1.3, and Student's paired t-test was used to analyse any differences found.

2.2.5.2 Results

Part 1: Effect of vigorous rubbing

An increase in OST was observed in all subjects after rubbing: the average change in OST was +0.21±0.16°C. Student's paired t-test (one tailed) showed the change approached significance ($p=0.08$).

Part 2: Effect of eye contact

Two subjects showed a no increase in OST after eye contact: the average change in OST was+0.06±0.39°C. Student's paired t-test (one tailed) showed the change was insignificant ($p=0.41$).

2.2.5.3 Conclusion

Despite the small numbers, the results indicate that procedures less invasive than eye contact such as insertion and removal of a contact lens and wiping away a tear should not significantly affect thermography results, but that extensive eye rubbing should be discouraged.

2.2.6
Effect of room temperature

Thermal gradient and equilibrium principles dictate that the rate at which the ocular surface will lose heat to the surrounding environment is related to the prevailing room temperature. Previous studies on OST have attempted to maintain stable room temperatures during experiments; some studies have examined changes in OST with room temperature:

- In rabbit eyes: 0.23°C change in ocular temperature accompanied a 1°C rise in environmental temperature (Schwartz, 1965)
- In 4 human subjects, measured 17 times over an 8 week period, a mean fall in OST of 0.145°C per degree decrease in environmental temperature over the range of 18-27°C was found (Mapstone, 1968b).
- Kolstad (Kolstad, 1970) demonstrated a reduction in corneal temperature (glass probe thermistor) with cold conditions (n=2).
- Two studies in the 70s observed an essentially linear decrease with ambient temperature drop, despite using very different methods (Freeman and Fatt, 1973; Rysa and Sarvaranta, 1974).
- Hørven showed a positive correlation between room temperature and corneal temperature using thermistors on 40 subjects (Horven, 1975)
- More recently, Hata and colleagues observed temperature decrease in cool conditions (Hata et al., 1994).
- Morgan observed the change in corneal temperature in one subject over a range of 21.7-30.4°C, and found a rate of change of 0.21°C per degree rise in room temperature (Morgan, 1994).

A strong correlation has been observed between body temperature and OST (see section 1.5.2.3), and there is a link between room temperature and body temperature (Colquhoun, 1971). As the temperature could be controlled within <±1°C in our laboratory set-up and there is strong previous evidence to suggest this will result in an ocular surface variation of <0.2°C no further experimentation over a range of temperatures was undertaken in this thesis.

2.3 Experimental Protocol

The review of the available literature and the studies in section 2.2 led to the development of a protocol for measurement of OST:

i. Subjects all attended at similar times of day (± 1 hour).
ii. Room temperature and humidity were controlled within ±1ºC/±1% and recorded.
iii. Subjects were allowed to adapt to room conditions for at least 10 minutes and they had to have been in the building for at least the previous 20 minutes.
iv. The average of at least three consecutive measures was calculated for a subject.
v. If repeated measures of the same subjects under different conditions were required, then a new 'baseline' recording was made at each visit for reference.
vi. Subjects were asked to blink normally and when instructed, to conduct one last normal blink, and then keep their eye open for eight seconds (time monitored by the examiner). A period of natural blinking (10-15 seconds) was encouraged in between recordings.
vii. Data collection and analysis was via Labview programming and Microsoft Excel (section 2.1.4). The term 'initial OST' refers to the temperature calculated just after a blink; other parameters recorded were the dynamic information about the change in OST post-blink (amount, rate). This data was recorded from twenty three points across the anterior eye, with the facility to be grouped into five main areas.

This protocol was used throughout the studies in this thesis.

CHAPTER 3

THE INFLUENCE OF PHYSICAL PROPERTIES OF THE ANTERIOR EYE AND THE TEAR FILM ON OST

3.1 Introduction

The source of the thermal radiation from the anterior eye is ambiguous and has been subject to assumptions about the thermal properties of the anterior eye (Chapter 1.3.2). It has been established that the media of the human eye absorb and emit infrared radiation efficiently, in a similar way to water (van den Berg and Spekreijse, 1997); particularly infrared wavelengths above 2.3µm (Lerman, 1980). The crystalline lens is known to have lower thermal conductivity and prevents significant heat flow from the posterior to the anterior regions of the eye (Scott, 1988). Studies have suggested that the measured temperature is essentially that of the tears (Hamano et al., 1969; Fatt and Chaston, 1980; Morgan et al., 1993), and only where the tears are absent will the radiation detected be that from the cornea itself: supported by a finite model of heat transport that calculates the temperature of the cornea to be less than that of tears (Scott, 1988). However, tear film thickness is still under debate (Korb et al., 2002) with values in the literature varying between 3µm (King-Smith et al., 2000) and 40µm (Prydal et al., 1992): Hamano's work suggests that a tear film thickness of 20µm or more will exhibit

100% absorption and emission of infrared radiation (i.e. the cornea will have little direct influence on measured radiation), whereas a tear film thickness of 10µm will absorb 80%, and 4µm will absorb 55% (Hamano et al., 1969). Thermal conductivity of the conjunctiva and sclera will be less than that of the cornea due to lower water content (Scott, 1988). It is logical to seek to further establish these ideas with the benefit of the latest thermo-camera technology.

Ocular temperature has been shown by several groups to correlate well with body temperature when measured aurally and orally (Schwartz, 1965; Schwartz et al., 1968; Mapstone, 1968b; Freeman and Fatt, 1973; Rysa and Sarvaranta, 1974; Horven, 1975; Morgan et al., 1993; Girardin et al., 1999), but there are few studies concerned with the influence of physical properties of the eye on OST as measured by infrared thermography.

Steeper corneas have been shown to have steeper thermal gradients across the ocular surface (Morgan et al., 1993), and negative correlations have been reported between corneal thickness and OST (Morgan, 1994; Du Toit et al., 1998). In cold stress studies, OST was shown to be slower to respond in eyes with deeper anterior chambers, and it was suggested that a greater volume of aqueous slows the internal effect of the fall in body temperature reaching the anterior eye (Rysa and Sarvaranta, 1973). However, a larger study showed no correlation between anterior chamber depth and OST (Morgan et al., 1993). Dry eye subjects have been shown to exhibit lower central OST and greater temperature gradients across the ocular surface compared to normals (Morgan et al., 1995). Rapid cooling of the tear film in dry eyes has been shown to related to reduced tear film stability and increased rate of evaporation (Morgan et al., 1996; Craig et al., 2000). There have been no reports of the use of tear meniscus height measurement in connection with OST, despite it being considered a useful subjective or objective assessment of tear volume (Korb, 2000).

The purpose of this study was to determine the relationships between the physical parameters (corneal thickness, corneal topography, bulbar hyperaemia and tear film stability) of the anterior eye and OST in a group of normals.

3.2 Methods

The right eyes of twenty-five young volunteers were used in this study (10 male, 15 female; mean age 27.1±3.8yrs). Inclusion criteria specified that all subjects had:

a) no history of eye disease
b) no contact lens wear for at least 48 hours
c) good general health and well-being
d) no dry eye symptoms (itchiness, grittiness, etc)

Subjects were requested not to wear eye make-up or to rub their eyes prior to or during the data collection. Full ethical approval from the university was granted prior to commencement of the study and informed consent was obtained from every subject. All procedures conformed to the tenets of the Declaration of Helsinki. Subjects attended randomly, for a single session, and measurements were made only on the right eye (as characteristics between eyes are known to be related), and in the same sequence for each subject:

Temperature

OST was measured using the Thermo Tracer TH7102MX, method as described in Chapter 2.1. Measurements were taken with (a) geometric corneal centre aligned with the camera, and (b) with the subject fixating on a target to their left so that the temporal bulbar conjunctiva aligned with the camera. Temperature measurements took place within a controlled environment (room temperature 21.4±0.2°C, humidity 39±1%. Subjects were required to room adapt for at least 10 minutes prior to imaging: during this period subjects practised the required blinking technique. The peak in OST following a blink, the change in OST over 8 seconds and the rate of such change was calculated. A reference to body temperature was taken by recording the radiated temperature on the forehead just above the root of the nose, for each subject (the recommended stable reference point by the International Medical Academy for Thermography (IMAT; www.imat.org/im/thn1us/litcrt1.htm).

Tear film & bulbar hyperaemia assessment

Digital images of the temporal bulbar conjunctiva, and the tear meniscus height (TMH) at centre of lower lid margin were recorded immediately after blinking using a slit-lamp biomicroscope camera system (Topcon SL7F with JVC KY F58 3CCD colour video camera). Resolution, illumination properties, and magnification were kept constant between subjects (x10 magnification, viewing angle 60° for bulbar conjunctiva; 40x magnification, 0° viewing angle for TMH). Custom designed software using Labview (NI Instruments, USA) allowed objective image analysis of the images to quantify bulbar hyperaemia, and (TMH) (Wolffsohn and Purslow, 2003). Non-invasive tear break-up time (NIBUT) was measured (5 repeated measures) using a Tearscope®: the break in the tear film was determined and timed when subjects are asked to blink and hold their eye open.

Corneal parameters

Average corneal curvature (anterior surface), corneal thickness and anterior chamber depth (ACD) were assessed using Orbscan II® (average of three scans calculated). The Orbscan II topography system (Bausch & Lomb) uses the lateral displacement of 2 slit beams and a video camera. It takes several images of different corneal sections for 3-dimensional reconstruction of corneal tissue, mapping the anterior and posterior corneal surfaces as well as the full corneal thickness. The anterior chamber depth is calculated from information about the position of the anterior lens surface. Orbscan was selected as the chosen method for corneal thickness measurement because it allowed corneal thickness to be calculated at locations that could be exactly corresponded with the temperature recording points of the thermo-camera (Chapter 2.1.3).

Given the findings from the literature (Chapter 1.4), the hypothesis was that initial OST and/or dynamic changes in OST following a blink would be most related to stability of the tear film and less related to physical characteristics of the anterior eye.

3.3 Results

Multiple regression analysis was used to calculate Pearson correlations between independent variables (NIBUT, TMH, conjunctival redness, ACD, corneal thickness, forehead temperature, average curvature/K) and the OST parameters measured (initial OST, and change in OST post-blink), for each area across the anterior eye. Standardised coefficients were calculated to compare the relative contribution of each independent variable, and then hierarchal regression analysis was used to explore the contribution of selected variables as appropriate.

For ease, the results have been grouped into initial OST (3.3.1), post-blink change in OST (3.3.2), and rate of post-blink change (3.3.3), and then the significant results presented by area.

3.3.1 Initial OST

There was a strong positive correlation between initial OST and forehead temperature (average 35.63±0.91°C) in all areas (Table 3.1).

AREA	Pearson Correlation with forehead temperature	Significance
CENTRAL	0.73	p<0.0005
NASAL	0.66	p<0.0005
SUPERIOR	0.71	p<0.0005
INFERIOR	0.72	p<0.0005
TEMPORAL	0.71	p<0.0005

Table 3.1
Correlations between initial OST and forehead temperature, in all areas (n=25)

In a regression model including all the parameters measured in the study, forehead temperature was the most significant influence on initial OST in all areas. Consequently, it was decided to remove this variable for subsequent regression modelling so that the influence of anterior eye parameters alone could be examined in each area.

Table 3.2 shows the correlation matrix between initial OST and the remaining independent variables, with the significant relationships highlighted. A negative correlation between corneal thickness and initial OST was observed in all areas but was not statistically significant. The correlation between thermal gradients (i.e. nasal OST minus central OST, and temporal OST minus central OST), and average corneal curvature was insignificant (nasally: r=0.23, p=0.26; temporally r=0.08, p=0.72)

			PEARSON CORRELATIONS WITH:				
VARIABLE	RANGE (n=20)	MEAN (±SD)	CENTRAL OST - (36.21±1.17°C)	NASAL OST - (36.50±1.10°C)	SUPERIOR OST- (36.40±1.19°C)	INFERIOR OST - (36.27±1.24°C)	TEMPORAL OST- (36.09±1.18°C)
NIBUT (seconds)	6.1 - 36.2	15.2± 8.4	-0.50*	-0.48*	-0.59*	-0.44*	-0.51*
TMH (mm)	0.18 -0.47	0.33± 0.09	-0.18	-0.21	-0.19	-0.18	-0.17
Bulbar hyperaemia (objective redness score)	0.336 - 0.392 Efron scale grade 0 - 2	0.36± 0.15	0.06	0.10	0.06	0.13	0.05
Anterior chamber depth (mm)	3.4 - 4.2	3.7± 0.2	0.07	0.01	0.08	0.13	0.11
Corneal curvature (mean K; dioptres)	40.4 - 47.1	42.9± 1.5	-0.41*	-0.38*	-0.36*	-0.40*	-0.41*
Central corneal thickness (µm)	476 - 606	548.7± 33.6	-0.26	-	-	-	-
Nasal corneal thickness (µm)	563 - 674	620.2± 34.1	-	-0.27	-	-	-
Superior corneal thickness (µm)	584 - 688	630.6± 34.3	-	-	-0.20	-	-
Inferior corneal thickness (µm)	517 - 630	588.2± 33.1	-	-	-	-0.20	-
Temporal corneal thickness (µm)	520 - 638	577.6± 40.4	-	-	-	-	-0.24

Table 3.2
Correlation matrix between OST in all areas and physical parameters of the anterior eye (n=25)
* indicates significant ($p<0.05$)

3.3.1.1 Central area

Negative correlations were found between initial central OST and NIBUT (r= -0.50, n=25, p<0.005), and average corneal curvature (r= -0.41, p<0.05; Figure 3.1).

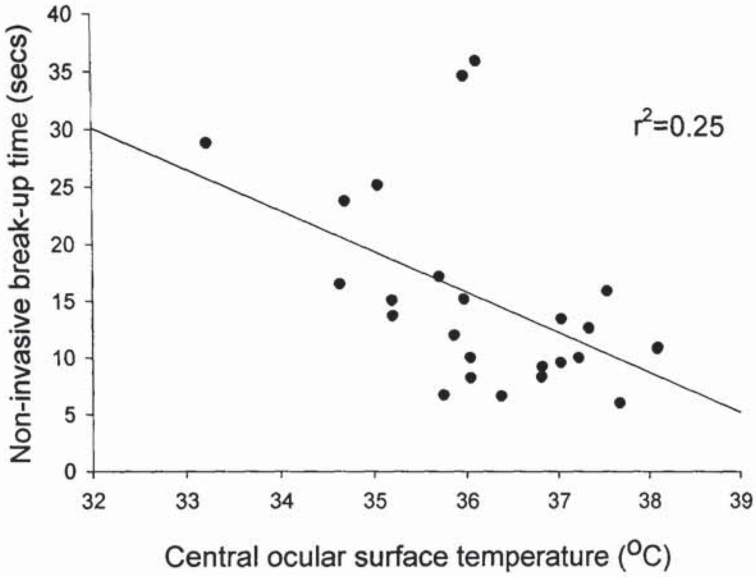
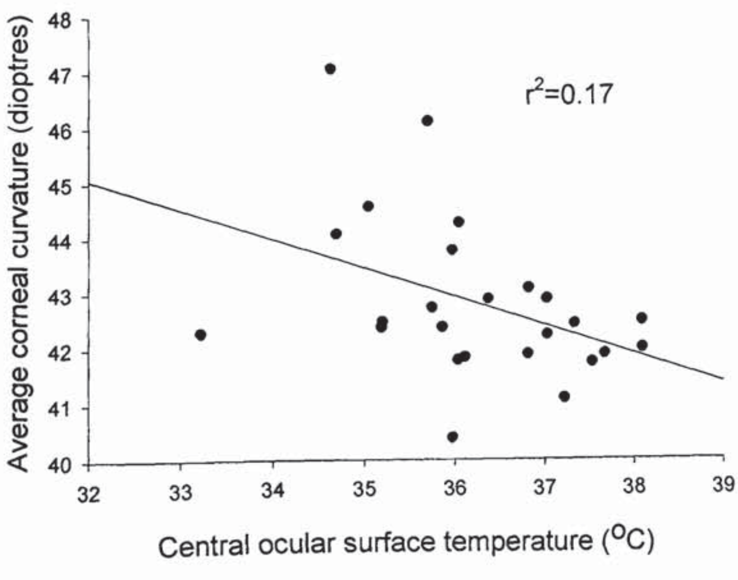


Figure 3.1
Correlation between initial central OST and NIBUT and average K



Initial OST in the central area was significantly correlated with the model including all the variables (r^2=0.50, F=2.96, p<0.05). Using hierarchal regression modelling, NIBUT (standardised coefficient= -0.51, p<0.01) accounted for 25.3% of the variance and average corneal curvature (standardised coefficient= -0.43, p<0.05) accounted for 17.4% of the variance in initial OST. When these two variables were controlled for, the remaining (central thickness, conjunctival redness, ACD and TMH, *in descending order of influence*) explained only 7.2% of the variance (p=0.73).

3.3.1.2 Nasal area

Similar individual correlations were found in the nasal limbal area between Initial OST and NIBUT (r= -0.48, n=25, p<0.01), and between OST and average corneal curvature (r= -0.38, n=25, p<0.05).

The complete group of variables accounted for 45.3% of the variance in OST but this only approached significance (F=2.95, p=0.06).

NIBUT (standardised coefficient=-0.48) and average corneal curvature (standardised coefficient=-0.40) and accounted for a significant 22.5% (p<0.05) and 15.8% (p<0.05) of the variance, respectively. The remaining variables accounting for 7.9% (p=0.63) and followed the same order of influence as for the central area.

3.3.1.3 Superior area

Again, in the nasal limbal area the only significant individual correlations with initial OST were observed with NIBUT (r= -0.59, n=25, p<0.005), and average corneal curvature (r= -0.36, n=25, p<0.05).

The complete group of variables accounted for 39.2% of the variance in OST (F=3.58, p<0.05).

NIBUT (standardised coefficient= -0.59) accounted for a significant 35.3% (p<0.005), and average corneal curvature (standardised coefficient= 0.38) accounted for 14.5% (p<0.05) of the variance. The remaining variables accounting for 4.5% (p=0.77): the individual influences being small and insignificant (in descending order: ACD, corneal thickness, redness, TMH).

3.3.1.4 Inferior area

Similar individual correlations were found in the inferior limbal area between Initial OST and NIBUT ($r = -0.44$, $n=25$, $p<0.05$), and between OST and average corneal curvature ($r = -0.40$, $n=25$, $p<0.05$).

The complete group of variables accounted for 45.5% of the variance in OST but this only approached significance ($F=2.51$, $p=0.06$).

NIBUT (standardised coefficient=0.45) and average corneal curvature (standardised coefficient=0.42) and accounted for a significant 19.1% ($p<0.05$) and 17.3% ($p<0.05$) of the variance, respectively. The remaining variables accounting for 9.1% ($p=0.57$); the descending order of influence being: ACD, redness, corneal thickness and TMH.

3.3.1.5 Temporal area

Negative correlations were found between initial temporal OST and NIBUT ($r = -0.51$, $n=25$, $p<0.005$), and average corneal curvature ($r = -0.41$, $p<0.05$).

Initial OST was significantly correlated with the variables ($r^2=0.50$, $F=2.98$, $p<0.05$). Using hierarchal regression modelling, NIBUT (standardised coefficient= -0.51, $p<0.01$) accounted for 26.1% of the variance and average corneal curvature (standardised coefficient=-0.42, $p<0.05$) accounted for 17.8% of the variance in initial OST. When these two variables were controlled for, the remaining (ACD, conjunctival redness, central thickness, and TMH, *in descending order of influence*) explained only 6.0% of the variance ($p=0.71$).

3.3.2 Post-blink change in OST

The change in OST over 8s after a blink was calculated for each area and the correlation between parameters analysed. The individual Pearson's correlations are shown in Table 3.3.

There was a significant negative correlation between NIBUT and magnitude of post-blink change in OST in the superior area ($r = -0.62$, $n=25$, $p<0.05$). No other significant relationships were observed in any of the remaining areas between the amount of post-blink cooling and the variables measured ($0.21>r^2>0.39$, $n=25$, $0.21>p>0.66$).

			PEARSON CORRELATIONS WITH AMOUNT OF POST-BLINK CHANGE IN OST, BY AREA:				
VARIABLE	**RANGE (n=20)**	**MEAN (±SD)**	**CENTRAL -0.96±0.93°C**	**NASAL -0.55±0.34°C**	**SUPERIOR -0.36±0.26°C**	**INFERIOR -0.35±0.38°C**	**TEMPORAL -0.40±0.37°C**
Forehead temperature (°C)	34.13 - 37.16	35.63± 0.91	-0.16	0.12	0.30	-0.14	0.19
NIBUT (seconds)	6.1 -36.2	15.2± 8.4	-0.27	-0.35	-0.62*	-0.15	-0.53
TMH (mm)	0.18 -0.47	0.33± 0.09	-0.16	-0.06	0.08	-0.20	-0.08
Bulbar hyperaemia (objective redness score)	0.336-0.392	0.36± 0.15	-0.06	-0.15	-0.27	-0.11	-0.30
Anterior chamber depth (mm)	3.4 - 4.2	3.7± 0.2	-0.10	-0.09	-0.20	0.13	0.09
Corneal curvature (mean K; dioptres)	40.4 -47.1	42.9± 1.5	-0.03	0.26	0.10	0.41	0.28
Central corneal thickness (µm)	476-606	548.7± 33.6	0.02	-	-	-	-
Nasal corneal thickness (µm)	563-674	620.2± 34.1	-	0.04	-	-	-
Superior corneal thickness (µm)	584-688	630.6± 34.3	-	-	-0.15	-	-
Inferior corneal thickness (µm)	517-630	588.2± 33.1	-	-	-	0.17	-
Temporal corneal thickness (µm)	520-638	577.6± 40.4	-	-	-	-	-0.08

Table 3.3
Correlations between amount of post-blink change in OST and the physical parameters of the anterior eye, in all areas (n=25)
* indicates significant ($p<0.05$)

3.3.3 Rate of Post-blink change in OST

Table 3.4 shows the correlation coefficients between the rate of temperature change over 8 seconds (using ½ life values, *i.e.* the time taken for ½ the amount of post-blink cooling to occur), and the other physical parameters measured in this study.

In central and temporal areas, the results suggested a significant positive correlation between NIBUT and the time taken to reach ½ life values ($r^2=0.16$, $p<0.05$ and $r^2=0.26$, $p<0.05$, respectively).

In the nasal area, objective redness score demonstrated a significant positive correlation with the time taken to reach ½ life values ($r^2=0.37$, $p<0.001$). In central, superior and inferior areas this relationship approached significance ($p=0.06$). Nasal corneal thickness also appeared significantly correlated ($r=0.41$, $p<0.05$), but within the regression model its effect was insignificant ($r^2=0.05$, $p=0.20$).

			PEARSON CORRELATIONS WITH THE RATE OF POST-BLINK CHANGE IN OST (represented by ½ life values), BY AREA:				
VARIABLE	**RANGE (n=20)**	**MEAN (±SD)**	**CENTRAL 2.04±1.05 sec**	**NASAL 1.81±0.72 sec**	**SUPERIOR 2.57±1.31 sec**	**INFERIOR 2.67±1.36 sec**	**TEMPORAL 2.46±1.04 sec**
Forehead temperature (°C)	34.13 - 37.16	35.63± 0.91	-0.06	-0.04	-0.03	0.05	-0.24
NIBUT (seconds)	6.1 -36.2	15.2± 8.4	0.40*	0.24	0.23	0.14	0.51*
TMH (mm)	0.18 -0.47	0.33± 0.09	-0.12	0.19	0.22	0.04	-0.04
Bulbar hyperaemia (objective redness score)	0.336-0.392	0.36± 0.15	0.33	0.61*	0.37	0.33	0.15
Anterior chamber depth (mm)	3.4 - 4.2	3.7± 0.2	-0.04	-0.07	0.14	-0.16	0.19
Corneal curvature (mean K; dioptres)	40.4 -47.1	42.9± 1.5	0.02	0.01	0.11	-0.01	-0.05
Central corneal thickness (µm)	476-606	548.7± 33.6	-0.11	-	-	-	-
Nasal corneal thickness (µm)	563-674	620.2± 34.1	-	0.41*	-	-	-
Superior corneal thickness (µm)	584-688	630.6± 34.3	-	-	0.29	-	-
Inferior corneal thickness (µm)	517-630	588.2± 33.1	-	-	-	-0.05	-
Temporal corneal thickness (µm)	520-638	577.6± 40.4	-	-	-	-	-0.12

Table 3.4
Correlations between the rate of post-blink temperature change in OST and physical parameters of the anterior eye, in all areas (n=25)
* indicates significant ($p<0.05$)

3.4 Discussion

The results support previous theories that OST measured by infrared thermography is principally influenced by the tear film and body temperature (Mapstone, 1968b). The strong correlation between body temperature and OST found in this study supports previous research (Schwartz, 1965; Schwartz et al., 1968; Mapstone, 1968b; Freeman and Fatt, 1973; Rysa and Sarvaranta, 1974; Horven, 1975; Morgan et al., 1993; Girardin et al., 1999).

The initial measure of OST (0.2s after a blink) appears to be negatively correlated with NIBUT (the stronger relationship), and average corneal curvature in all areas across the anterior eye.

Eyes with steeper corneas appeared to be cooler centrally: possibly the blink action is less efficient at spreading warm tears over a steeper, more 'exposed' cornea: where tears are thinner the temperature recorded will be closer to that of the cornea, which should be cooler than that of tears (Hamano et al., 1969; Scott, 1988). This study demonstrated a similar relationship between nasal thermal gradient and corneal curvature to an earlier larger study (r=0.23 compared with r=0.28 (Morgan et al., 1993), but it was not significant statistically. Weak correlations were also observed between corneal curvature and the amount and rate of post-blink change, but these were not statistically significant. This may relate to a discrepancy between when the OST is measured and the scanning technique of Orbscan: blinking and the resultant tear layer distribution can significantly influence topographic images (Nemeth et al., 2001).

Eyes with lower initial OST tended to have longer tear break-up times, i.e. a more stable tear film. This appears at odds with a previous suggestion that OST will be less in dry eye patients with decreased tear film stability due to deficient lipid layer (Craig et al., 2000). However, in that study OST was recorded 4-5 seconds following a blink rather than immediately (0.2s), as in this study, and dry eyes have been shown to cool rapidly after a blink (Morgan et al., 1996). Another study has described dry eyes with greater OST on eye opening (Morgan et al., 1996). A lower initial OST may result from a more stable tear film having different emissivity properties to a less stable one, possibly due to lipid content, *or* conversely, a longer break-up time may result from a cooler eye having slower rates of heat exchange via radiation and evaporation (Newton's Law of Cooling (Lerner, 1996). The latter idea is supported by the results from this study that suggest a tendency for eyes with longer NIBUT to exhibit smaller *and* slower temperature changes over the eight seconds following a blink. A fall in OST following a blink (as the warm tears thin and evaporate) is widely accepted (Efron et al., 1989; Morgan, 1994; Morgan et al., 1995), so it might be anticipated that increased tear film stability (longer NIBUT) will produce a smaller and slower change in OST between subjects. Previous work has found correlations between quality of the tear film and cooling post-blink: lipid-deficient dry eyes (increased evaporation) showed faster cooling (Morgan et al., 1996; Craig, 1997), and aqueous-deficient dry eyes demonstrated slower cooling (Mori et al., 1997). In this study, positive correlations were observed between time taken to reach ½ life values and NIBUT, i.e. as NIBUT increased, the time taken for cooling to occur tended to increase.

The results show little support for the idea that ocular temperature increases when blood flow to the anterior eye increases, or when the anterior eye is inflamed (Mapstone, 1968b, 1968d; Mikesell, 1978; Morgan et al., 1993). Previously, a positive correlation has been demonstrated with the McMonnie scale of bulbar redness (Efron et al., 1988), and between maximum OST and duration of ciliary injection (Mapstone, 1968b). This may have arisen because the range of redness in the subjects in this study was narrow, even though a sensitive technique of objective grading was used (Wolffsohn and Purslow, 2003). The results suggested a positive correlation between bulbar redness and the time taken to reach ½ life values in the nasal area: possible reasons for this may be a prolonged warming effect due to such hyperaemia, as well as possible uneven surface to hyperaemic areas of the conjunctiva.

Although not statistically significant, the trend for a negative correlation between corneal thickness and initial OST was seen in all areas, supporting previous findings (Morgan, 1994; Du Toit et al., 1998).

The number of reports about the influence of anterior chamber depth (ACD) on OST are few and conflicting (Rysa and Sarvaranta, 1974; Morgan et al., 1993): the results from this study show no significant relationship.

TMH has been considered by some workers to be an indirect measure of tear volume (Doughty et al., 2002), and subjective estimation of the tear meniscus has been considered a determinant of tear film stability (Khurana et al., 1991). It is perhaps surprising that no significant correlation between TMH and initial OST or post-blink change in OST, was observed in this study. However, some subjects in this study exhibited a TMH>0.3mm which could indicate slight reflex tearing (Doughty et al., 2002), possibly as a result of the illumination necessary for photography.

3.5 Conclusions

The results provide further information about the source of OST measured by infrared thermography: they help to further establish the idea that OST measured by infrared thermography is principally that of the tear film and that it will be related to parameters such as corneal curvature, rather than corneal thickness and anterior chamber depth.

CHAPTER 4

DYNAMIC OBSERVATIONS OF OST

4.1 Introduction

Chapter 3 added further support to the model of OST being principally influenced by the tear film. As such, OST measured by infrared thermography will be affected by the dynamic nature of the tear film (Mapstone, 1968b; Craig et al., 2000), and it has been recognised that the ability to study OST in real time would be advantageous (Morgan et al., 1995). In previous studies, measurement of OST has taken the form of a static value taken at a certain time after eye opening (Alio and Padron, 1982a; Efron et al., 1989; Morgan et al., 1993; Girardin et al., 1999). As the eyelid closes, the ocular surface is juxtaposed with the vascular palpebral conjunctiva and exposed to fresh tears from the lacrimal gland. An initial rise in OST followed by a rapid decrease as heat is lost by convection and radiation has been observed; the initial temperature peak seems to be greater with forceful blinking (Morgan, 1994): lacrimal secretion increases under forced blink conditions (Doane, 1981) and the globe rotates upwards during a long blink compared to a short one (Vandersteen et al., 1984).

Quantifying the rate of cooling in OST after a blink has previously been limited to a series of static measures over prolonged periods of forced open eye conditions, due to the prevailing technology (Mapstone, 1968b; Efron et al., 1989). Subjects whose 'corneas' cooled more slowly had greater inter-blink intervals (Efron et al., 1989). The cornea possesses specific thermal receptors and it has been suggested that the detection of the tear film cooling is part of the blink process (Murphy et al., 1999; Murphy et al., 2001). Morgan and colleagues

(Morgan et al., 1996) were able to show that the rate of cooling in OST was greater in 'dry eyes' (static measures taken every second over an 8 second period). Greater values for initial mean OST (across the *entire* ocular surface) and increased rates of evaporation observed in dry eyes were suggested as explanations for this observation.

The existence of a characteristic thermal profile across the ocular surface is well-established (Alio and Padron, 1982a; Efron et al., 1989; Morgan et al., 1993), but temporal changes in this profile have received little attention. It has been observed that dry eyes generally show greater differences in OST between central and limbal areas that are less stable (Morgan et al., 1995; Craig et al., 2000). Rationalising the thermal profile has previously centred on the differential warming of the tear film by adjacent ocular structures, but there appears to have been little attention to the influence of tear flow dynamics on OST profiles in the literature. Novel techniques such as high speed video (Owens and Phillips, 2001) and videotopographic systems (Nemeth et al., 2002; Goto et al., 2003) have been used to quantify tear film spreading rates but no connection has been made with dynamic changes in OST.

No significant gender effect on static measures of OST has been observed in previously (Schwartz et al., 1968; Horven, 1975; Alio and Padron, 1982b; Morgan, 1994), but these studies employed a wide age range of subjects. Gender differences in tear film physiology exist according to the age of subjects considered: a lower tear osmolality has been observed in young females compared to young males, attributed to increased tear flow (reflex secretion) leading to relatively hypotonic tears (Craig and Tomlinson, 1995). No significant gender differences in evaporation or stability have been found (Craig and Tomlinson, 1998; Patel et al., 2000). Dynamic measures of OST post-blink with respect to gender have not yet been examined.

This study was designed to examine dynamic changes in OST across the ocular surface in a large sample of the population using dynamic infrared thermography, and to relate such findings to relevant ocular physiology.

4.2 Methods

The right eyes of 200 young volunteers were used in this study (85 male; 115 female; mean age 26.6±4.1yrs). Inclusion criteria specified that all subjects had:

a) no history of eye disease
b) no contact lens wear for at least 48 hours
c) good general health and well-being
d) no dry eye symptoms (itchiness, grittiness, etc)

Full ethical approval from the university was granted prior to commencement of the study and informed consent was obtained from every subject. All procedures conformed to the tenets of the Declaration of Helsinki. Subjects were requested not to wear eye make-up or to rub their eyes prior to or during the data collection. Subjects attended randomly, for a single session, and between 10am and 12noon: section 2.2.3 demonstrated no significant differences in OST measurements taken at these times (p=0.36). In accordance with the conclusions of section 2.2, each subject was allowed to adapt to room temperature for at least 10 minutes before OST of the right eye was recorded 3 consecutive times. Experimental set-up and collection of data was as previously described (section 2.1): room temperature and humidity were controlled at 21±0.6°C and 40±2%. Subjects were required to blink normally and hold their eye open for eight seconds on measurement (easily achieved by subjects). Data collection and analysis was as described in section 2.1.4. The initial OST, the decrease in OST over the eight seconds and the time taken to reach ¼, ½ and ¾ of this decrease, were calculated in order to describe the dynamic profile of the OST across the anterior eye surface (figure 4.1).

Mean values for these parameters of OST were calculated for the five areas and examined for correlations and differences: Pearson's correlation coefficient and analysis of variance were used. Gender differences were also compared using non-parametric statistical testing (Mann-Whitney U test). Differences between central and nasal & temporal areas were calculated in order to describe nasal & temporal *thermal gradients*.

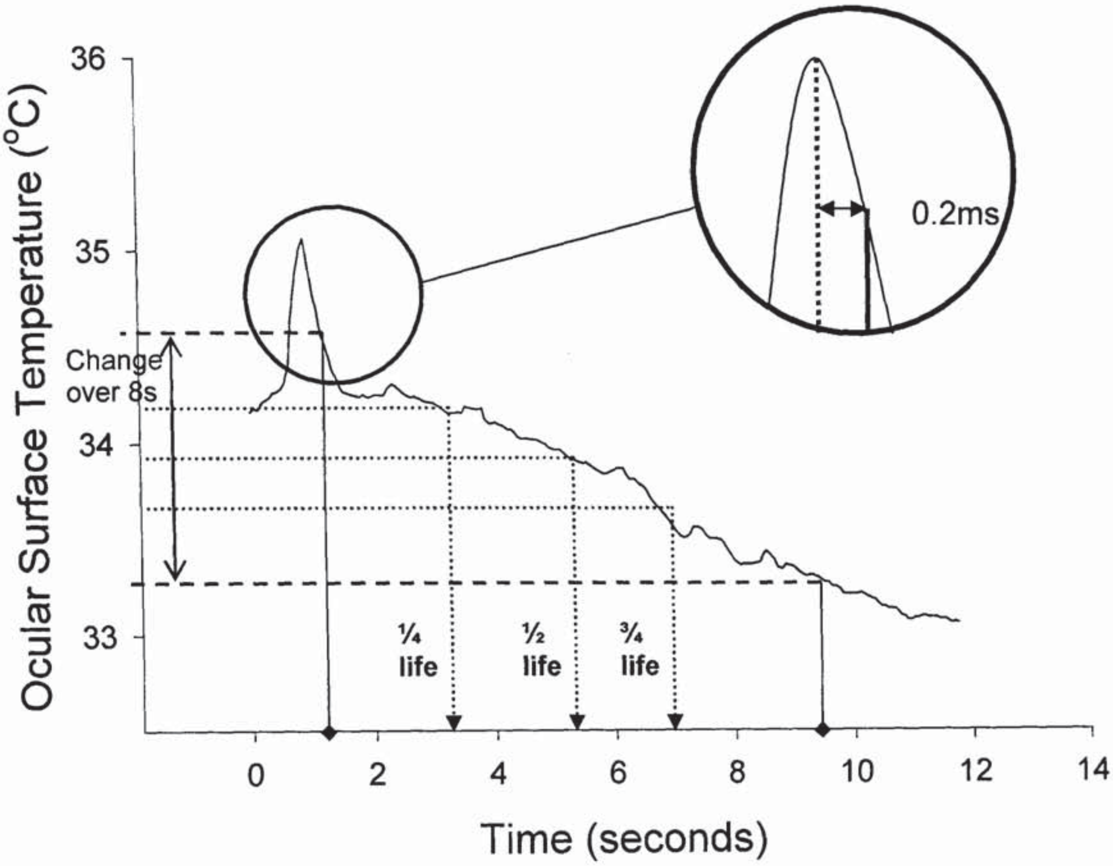


Figure 4.1
Typical temporal change in OST with blinking, showing reference points selected for analysis

4.3 Results

Table 4.1 shows the mean results for parameters of OST across five areas of the ocular surface: initial OST (0.2s after a blink), change in OST following a blink (after 8s), and the time taken to achieve ¼, ½ and ¾ of the post-blink temperature change.

AREA	Initial OST (0.2s after a blink) (°C ± 1sd)	Change in OST over 8s following a blink (°C ± 1sd)	Rate of decrease in OST post blink (seconds±1sd)		
			¼ of post-blink change	½ of post-blink change	¾ of post-blink change
CENTRAL	36.67 ±1.3	-0.68 ±0.5	0.9±1.0	1.7±1.1	3.5±1.2
NASAL	37.01 ±1.3	-0.44 ±0.4	1.3±1.3	2.1±1.4	3.7±1.2
SUPERIOR	36.71 ±1.4	-0.44 ±0.5	1.6±1.6	2.4±1.6	3.7±1.5
INFERIOR	36.71 ±1.4	-0.24 ±0.5	1.9±1.7	2.8±1.7	4.1±1.5
TEMPORAL	36.66 ±1.5	-0.40 ±0.5	1.8±1.6	2.7±1.7	4.2±1.2

Table 4.1
Mean OST parameters in five regions of the ocular surface of the right eye (n=200)

4.3.1 Initial OST

Central and temporal limbal areas appeared coolest, and the nasal limbal area warmest, but there was no statistically significant difference in initial OST across the five areas of the ocular surface ($F=2.15$; $p=0.07$). Figure 4.2 shows the frequency distribution of initial OST (n=200), for the five regions of the ocular surface. Kolmogorov-Smirnov statistics confirmed normality of the distributions in all areas ($p=0.20$). Initial OST of the central area was strongly correlated with other regions of the anterior eye ($0.95<r^2<0.98$; Figures 4.3 & 4.4).

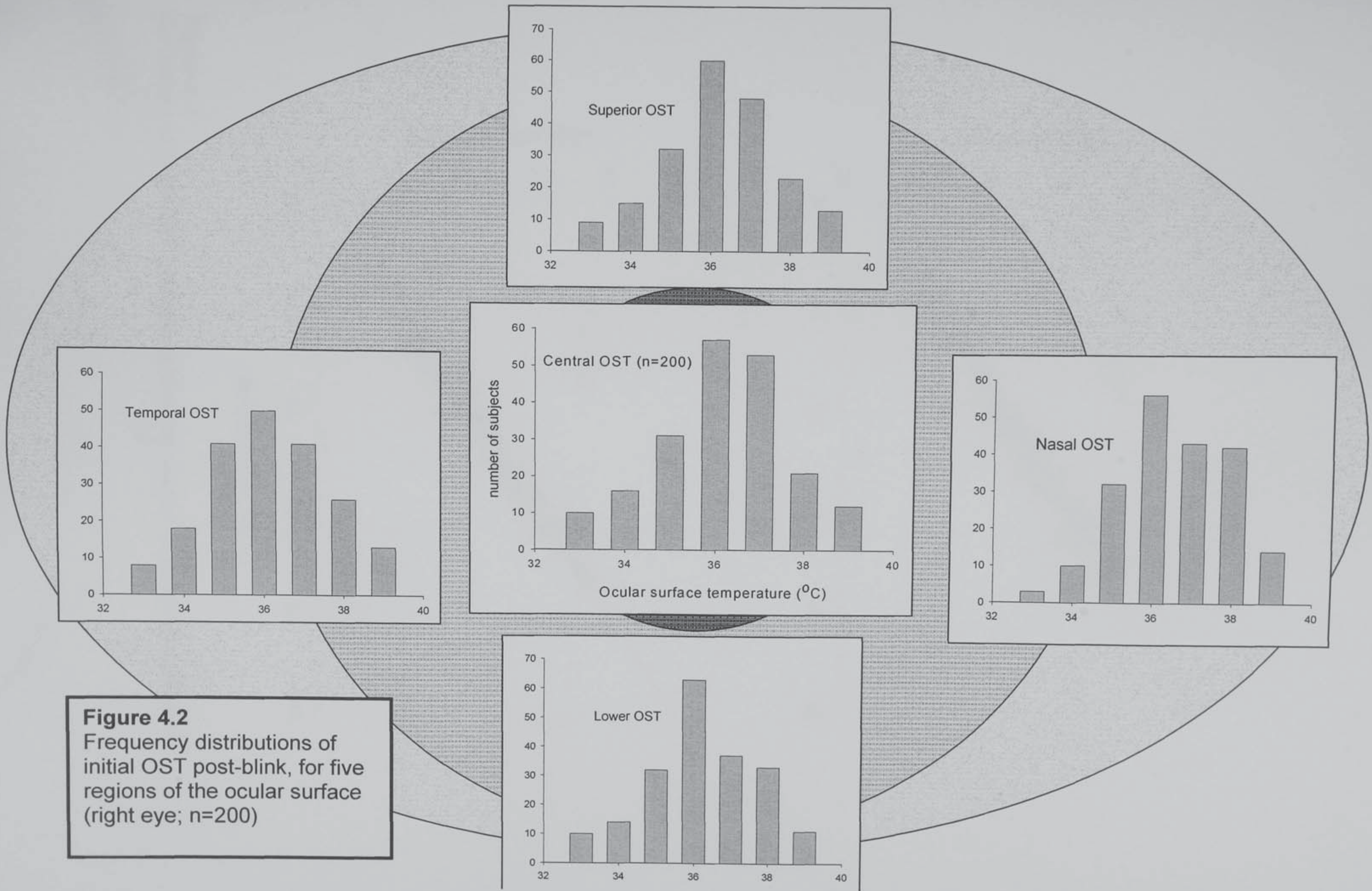
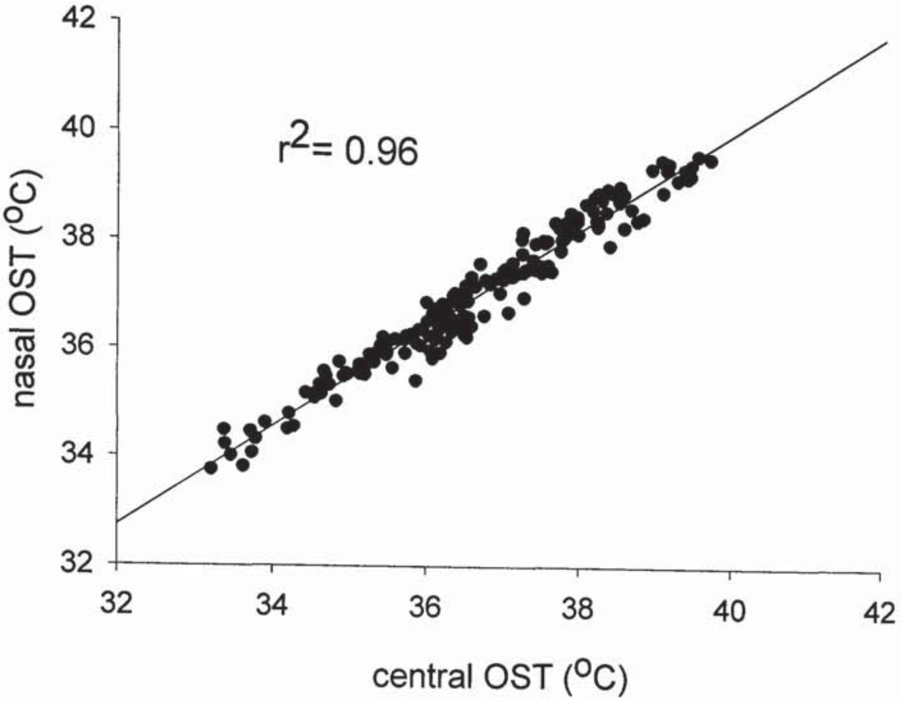


Figure 4.2
Frequency distributions of initial OST post-blink, for five regions of the ocular surface (right eye; n=200)



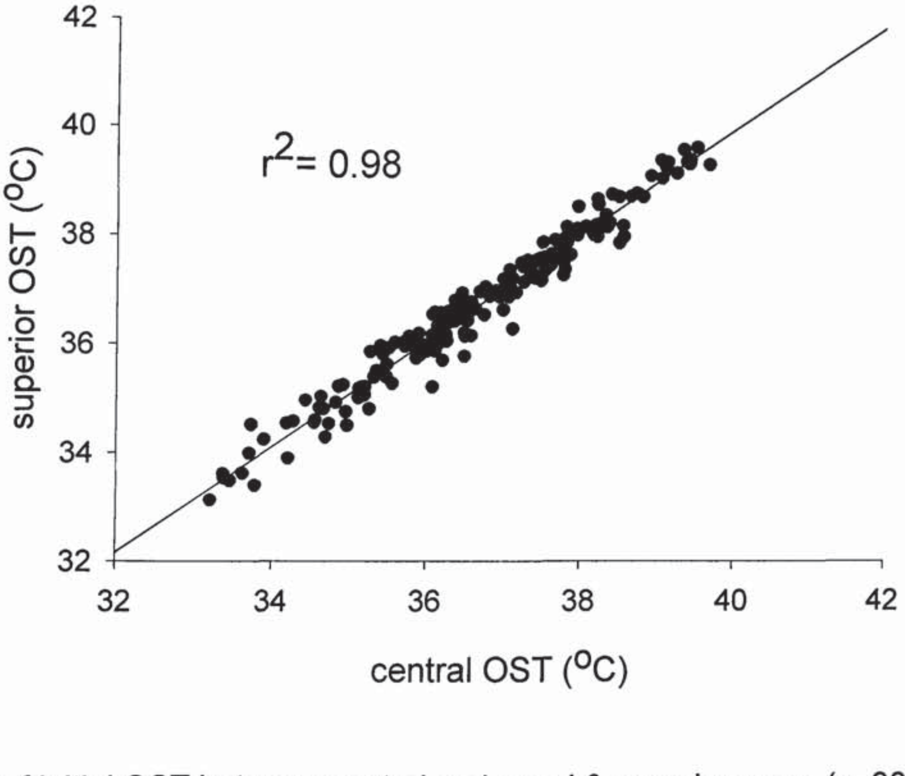


Figure 4.3
Correlation of initial OST between central and nasal & superior areas (n=200)

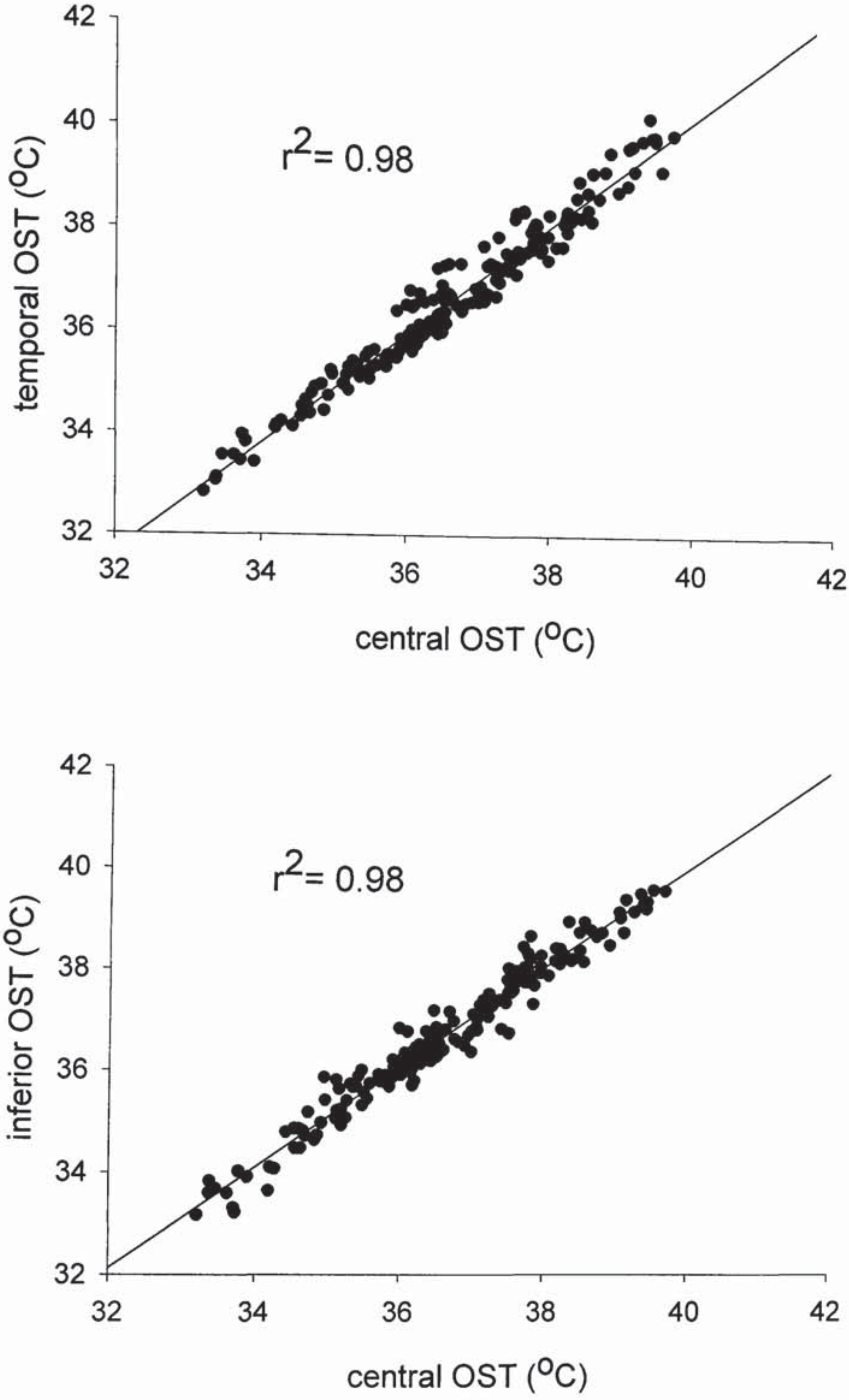


Figure 4.4
Correlation of initial OST between central and temporal & inferior areas (n=200)

4.3.2 Post-blink OST, after 8 seconds

A significant decrease in OST 8 seconds after a blink was observed in all areas (Student's paired t-test: 7.15<'T-value'<18.55; <p<0.0005). The central OST after 8 seconds was still strongly correlated with all other areas (0.94<r<0.98). The amount of post-blink decrease was significantly different between areas (ANOVA: F=21.66, n=200, p<0.0001), with the central area showing the greatest decrease after 8 seconds (Scheffe; p<0.0001 – Table 4.2 & Figure 4.5).

Region of ocular surface	Mean decrease in OST over 8 seconds (°C ± 1sd)	Mean ocular surface temperature, 8 seconds after a blink (°C ± 1sd)
CENTRAL	-0.68 ±0.5°C	35.99 ±1.4°C
NASAL	-0.44 ±0.4°C	36.57 ±1.4°C
SUPERIOR	-0.44 ±0.5°C	36.27 ±1.5°C
INFERIOR	-0.24 ±0.5°C	36.47 ±1.4°C
TEMPORAL	-0.40 ±0.5°C	36.26 ±1.6°C

Table 4.2
OST across the ocular surface, eight seconds after a blink (n=200)

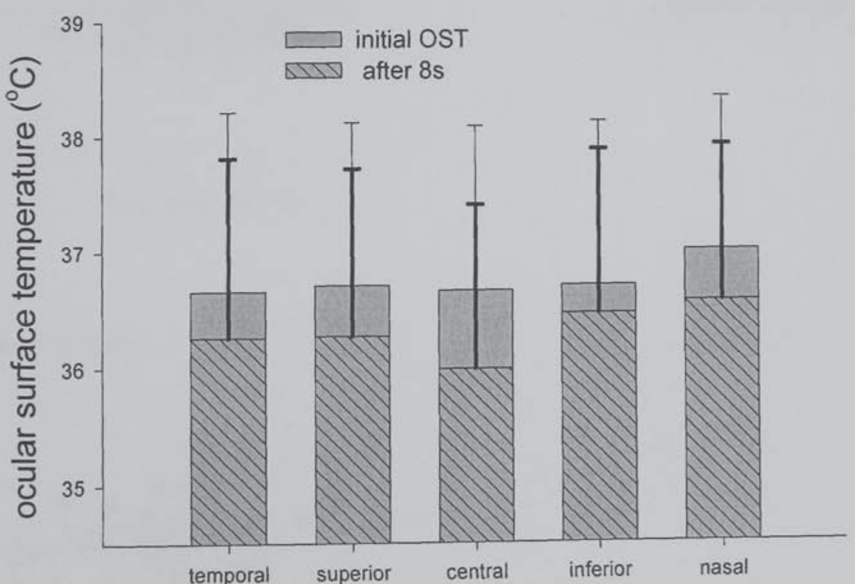


Figure 4.5
Change in OST over 8seconds following a blink, across the areas of the ocular surface (n=200)

There was a weak correlation between the initial OST and the magnitude of change in OST over 8 seconds, in any of the five areas ($0.05<r<0.20$; Figures 4.6 & 4.7).

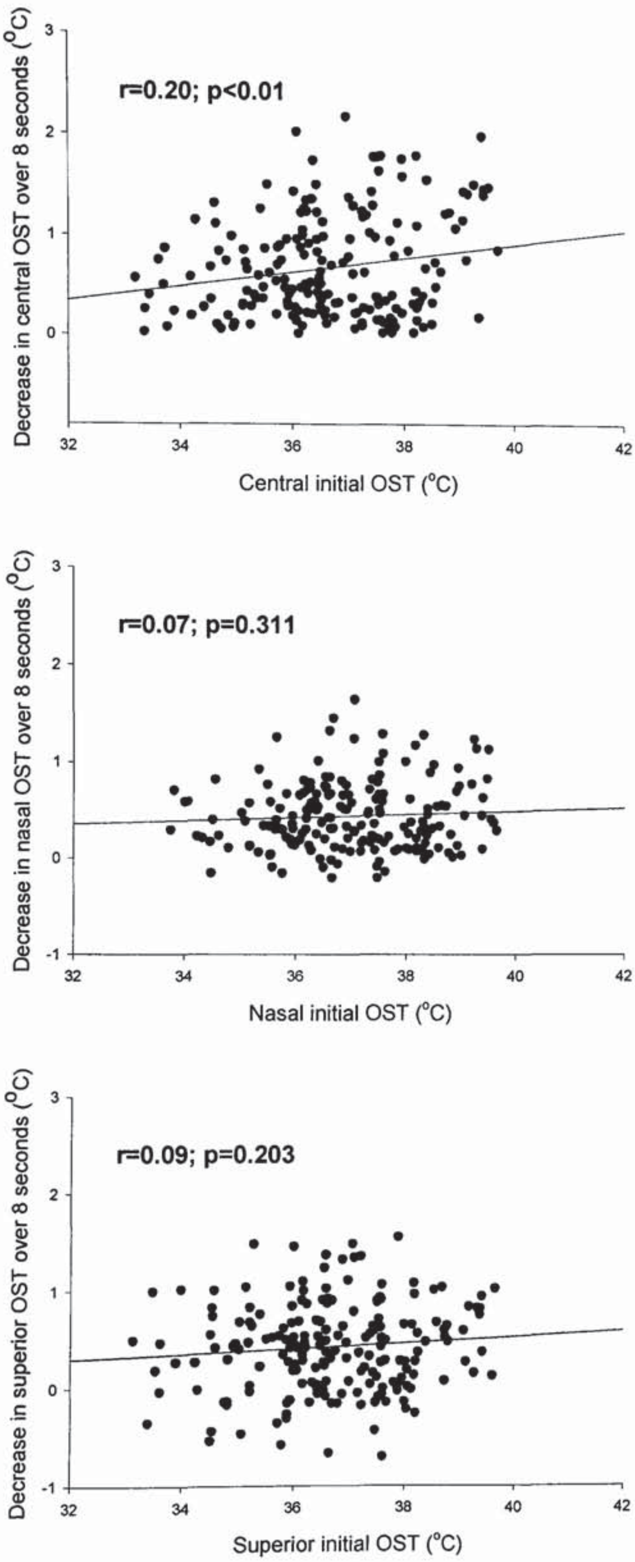


Figure 4.6
Correlation between initial OST and decrease over 8 seconds, for central, nasal and superior areas (n=200)

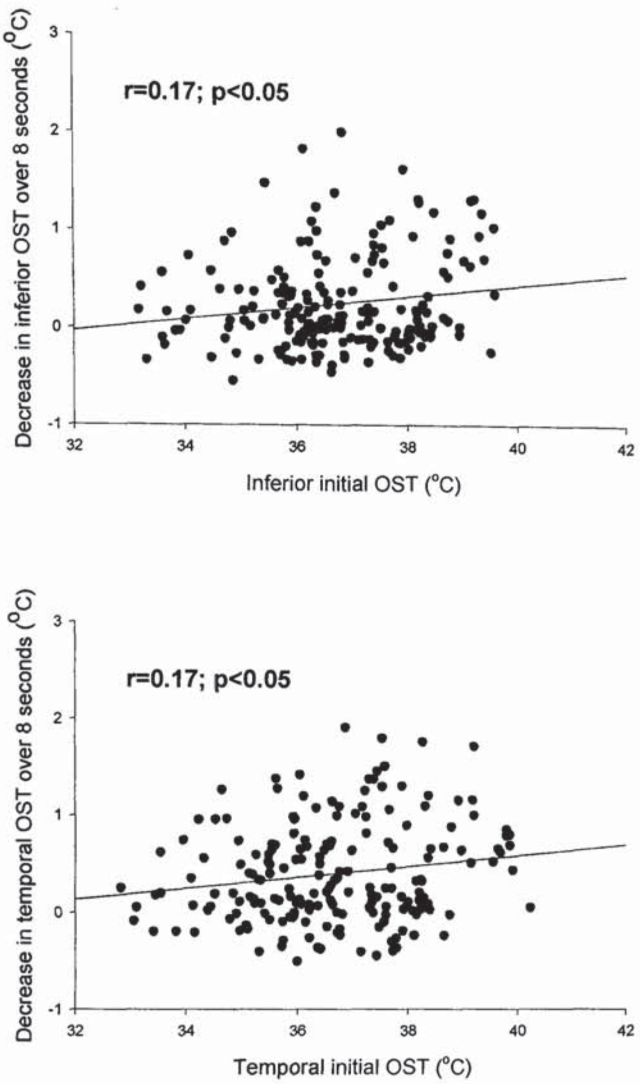


Figure 4.7
Correlation between initial OST and decrease over 8 seconds, for inferior and temporal areas (n=200)

4.3.3 Horizontal thermal profile

Immediately after a blink, only the OST in the nasal limbal area was significantly warmer than central OST (t-test t=2.51, p<0.05). Eight seconds later, both the difference from centre to nasal, and that from centre to temporal had increased (p<0.0005) to significant levels (Table 4.3). Nasal OST was significantly greater than temporal OST after 8 seconds (p<0.05).

	Mean difference between central and nasal OST (°C ± 1sd; sig.)	**Mean difference between central and temporal OST (°C ± 1sd; sig.)**
INITIAL OST	0.35±0.3°C; *p<0.05*	-0.01±0.3°C; *p=0.95*
AFTER 8 SECONDS	0.58±0.8°C; *p<0.0001*	0.27±0.4°C; *p<0.05*

Table 4.3
Initial and post-blink differences in OST between centre and nasal/temporal areas (n=200)

4.3.3 Vertical thermal profile

Immediately after a blink, mean OST over the central, superior and inferior areas was similar (ANOVA, F=0.65, p=0.94). After 8 seconds, OST was significantly different between centre and superior and inferior, with the most significant difference between central and inferior areas (Table 4.4).

	Mean difference between central and inferior OST (°C ± 1sd; sig.)	**Mean difference between central and superior OST (°C ± 1sd; sig.)**
INITIAL OST	0.04±0.3°C; *p=0.76*	-0.05±0.3°C; *p=0.75*
AFTER 8 SECONDS	0.48±0.4°C; *p<0.001*	0.28±0.5°C; *p<0.001*

Table 4.4
Initial and post-blink differences in OST between centre and superior/inferior areas (n=200)

4.3.5 Dynamic changes in OST following a blink

The rate of cooling over 8 seconds was calculated for each area (table 4.5); analysis of variance revealed significant differences between areas (F=21.65. p<0.0005), and post-hoc comparisons indicated that central and inferior areas were significantly different from the remaining (Scheffe: p<0.0005 and p<0.05 respectively).

	Rate of temperature change over 8 seconds (°C.sec^{-1}±1sd)
CENTRAL	0.084 ± 0.06
NASAL	0.055 ± 0.04
SUPERIOR	0.055 ± 0.06
INFERIOR	0.031 ± 0.06
TEMPORAL	0.050 ± 0.06

Table 4.5
Post-blink rate of cooling for five areas of the ocular surface (n=200)

The times taken to reach ¼, ½ and ¾ of the post-blink temperature change were compared for each area (Figures 4.8-4.10). Significant differences in the rate of cooling were observed between the five areas ($p<0.0001$; $F_{1/4}=15.81$; $F_{1/2}=16.20$; $F_{3/4}=103.40$): the central area demonstrated the quickest decrease in OST following a blink, whilst the inferior and temporal areas were slowest (Scheffe; p<0.0001).

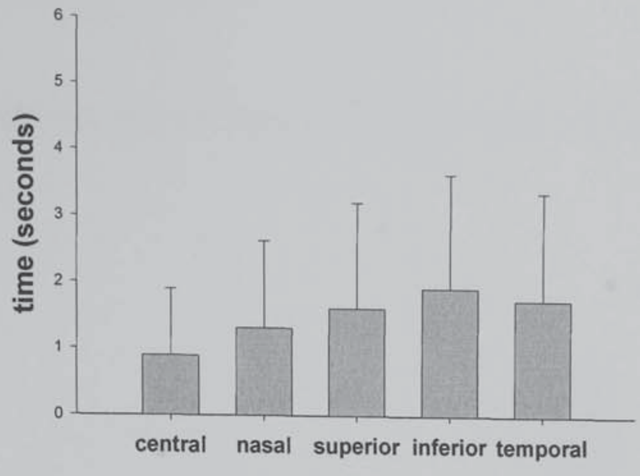


Figure 4.8 Time taken to reach ¼ post-blink temperature changes

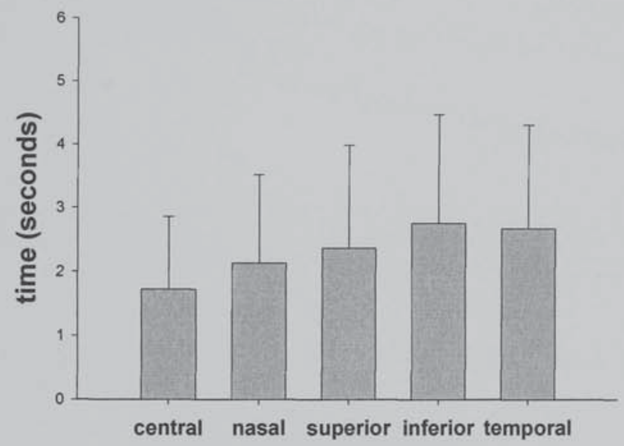


Figure 4.9 Time taken to reach ½ post-blink temperature changes

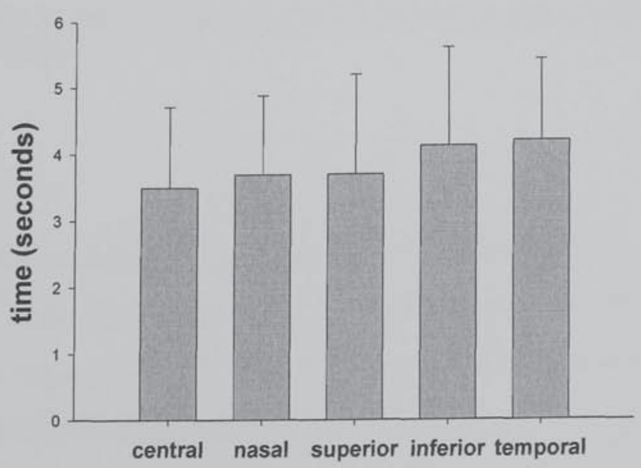


Figure 4.10 Time taken to reach ¾ post-blink temperature changes

Figure 4.11 illustrates the slope of this cooling following a blink, for the five areas of the ocular surface.

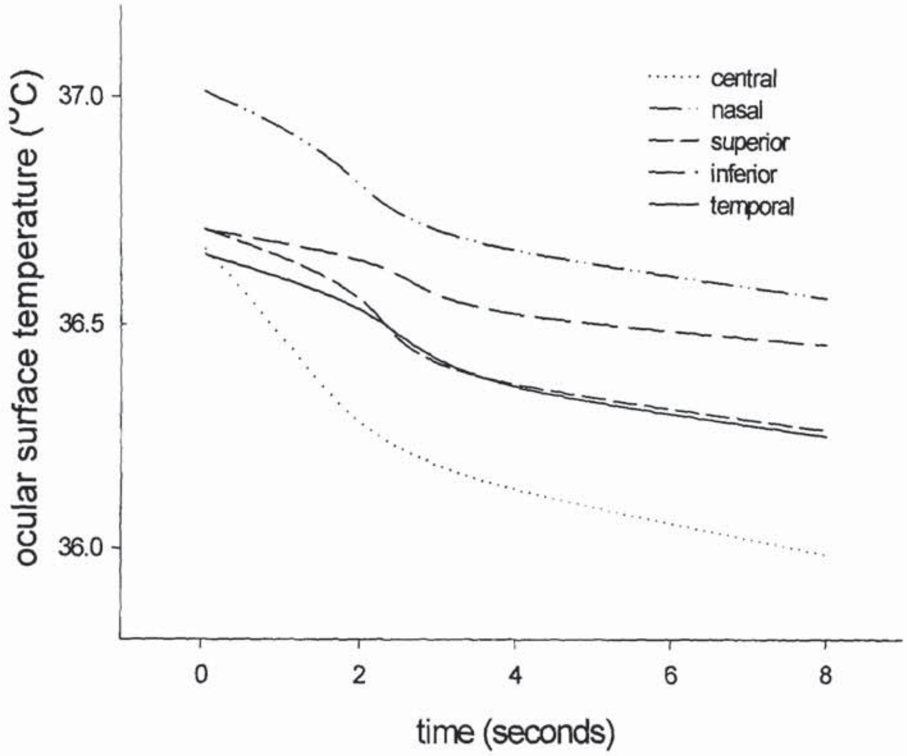


Figure 4.11
Mean change in OST in five areas across the ocular surface, for eight seconds following a blink (n=200)

There was little correlation (r=0.04; p=0.53) between the initial central OST and the rate of cooling post-blink (Figure 4.12); correlations in other areas were similar: r_{nasal}=0.05 (p=0.50); $r_{superior}$ =0.005 (p=0.99); $r_{inferior}$ =-0.05 (p=0.52). Greater initial OST in the temporal area correlated with faster cooling: $r_{temporal}$ =-0.18 (p<0.01).

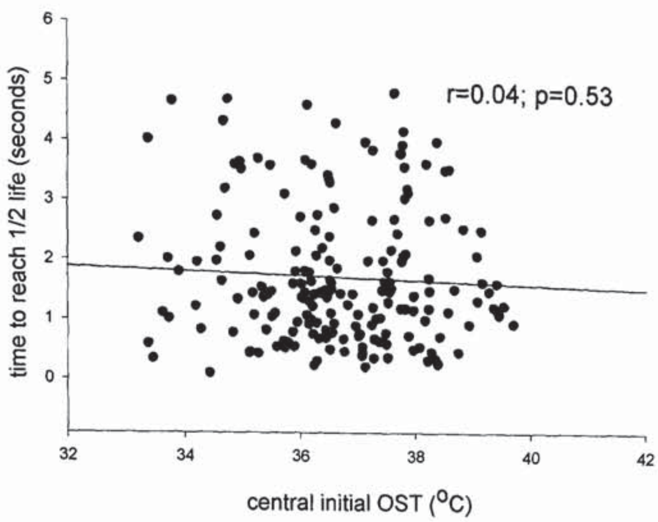


Figure 4.12
Correlation between initial OST (central) and rate of cooling (n=200)

As expected, a stronger relationship was observed between the rate of cooling and the quantity of such cooling over 8 seconds (Figure 4.13; $p<0.0001$). In other areas, this relationship was similar: $r_{nasal}= -0.50$ ($p<0.0001$); $r_{superior}= -0.41$ ($p<0.0001$); $r_{inferior} = -0.33$ ($p<0.0001$); $r_{temporal} = -0.37$ ($p<0.0001$).

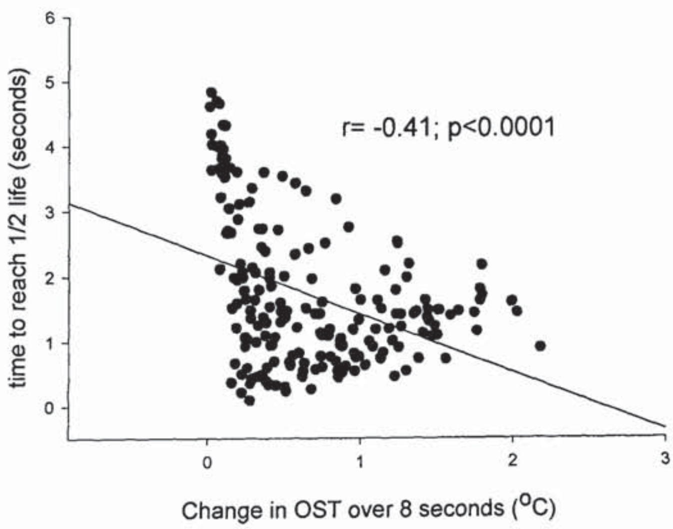


Figure 4.13
Correlation between change in OST post-blink and the rate of such change (central area; n=200)

4.3.6 Gender differences

No significant gender difference in initial OST was found between males and females (centrally: 36.72±1.2°C vs. 36.62±1.6°C; Mann-Whitney U test, $p_{central}=0.38$). Similar results were found for other areas: $p_{nasal}=0.63$ $p_{superior}=0.60$ $p_{inferior}=0.17$ $p_{temporal}=0.17$.

After 8 seconds, there were significant gender differences in OST: in temporal and inferior areas, females had significantly cooler OST (Mann Whitney $p<0.05$). In the central area the difference approached significance (males: 36.19±1.8°C vs. females: 35.84±2.1°C; Mann-Whitney U, $p=0.06$). The amount of post-blink change in OST was significantly greater for females, in all areas (Mann Whitney $p<0.0005$). Similarly, the rate of cooling was significantly faster in females, for all areas (Mann Whitney $p<0.0005$).

4.4 Discussion

4.4.1 Initial OST after a blink

The initial mean OST for the central area indicated by this study would at first inspection appear greater than that of most other infrared thermography studies: Efron and colleagues (Efron et al., 1989) obtained an average OST of 34.3ºC (n=21) at geometric corneal centre, two seconds after a blink; two larger studies demonstrated a central OST range of 32-34.5ºC (n=96) measured four to five seconds after a blink (Alio and Padron, 1982a; Morgan et al., 1993); Girardin and colleagues found OST to be 33.7±0.6ºC two seconds after a blink in 226 subjects (Girardin et al., 1999). Differences between this study and others appear to relate to the time point selected for measurement. An initial rise in OST is seen straight after a blink (closing the eyelids exposes the ocular surface to the vascular tarsal conjunctiva and fresh tears form the lacrimal gland), followed by a rapid decrease as heat is lost by convection and radiation (Efron et al., 1989). In this study, initial values for OST could be analysed as quickly as 0.2s following maximal temperature (the passing of the eyelid), and are therefore greater than previous work. At four seconds, mean OST for the central area was approximately 36.1ºC, which is still greater than other studies, but this figure is actually an average of nine points around and including the geometric centre of the cornea (covering approximately 16mm^2), which results in a higher temperature measurement compared to the single point/ small box (1mm^2) technique used in the earlier studies (Morgan et al., 1993). Also, all subjects in this study were young and OST has previously been shown to decrease with age (Rysa and Sarvaranta, 1973; Horven, 1975; Alio and Padron, 1982b; Girardin et al., 1999; Morgan et al., 1999).

The wide variability in absolute values of OST seen here is likely to result from individual variations in a variety of parameters including:

i. The strength of a 'normal blink': forceful blinking has been shown to produce a greater peak temperature rise upon opening the eyelids and faster cooling, whereas flick blinking a lesser effect (Morgan, 1994).

ii. Tear secretion rates: total tear volume within the palpebral aperture has been estimated at between 7-10μl (Maurice, 1973).

iii. Body temperature: the generally accepted standard of 37.0°C is surprisingly unsubstantiated in the literature, and a much wider range of 33.2-38.2°C can actually be found in a normal population (Sund-Levander et al., 2002). Location of measurement, gender and age are important (Mackowiak et al., 1992).

iv. Variation in corneal curvature between subjects. In Chapter 3, a negative correlation was observed between OST and corneal curvature.

The similarity in initial temperature across the five areas was expected, again due to the time-point of measurement: the blink action will have brought the tear film and anterior eye into intimate contact with the vascularised palpebral conjunctiva, such that immediately after its passing, radiated temperature from the eye will be more uniform. The marginally warmer OST over the nasal limbal area may result from its proximity to the nasal canthus, being a highly vascularised area.

4.4.2 Change in OST following a blink

The temperature results after 8 seconds support the presence of a characteristic thermal profile (where the bulbar areas of the anterior eye always appear warmer than central), which is well-established in the literature (Fielder et al., 1981; Alio and Padron, 1982a; Efron et al., 1989; Morgan et al., 1993). After 8 seconds the nasal and temporal differences found here were similar to those found in such studies, but here the nasal limbal area was found to be significantly warmer than temporal area, unlike a large previous study (Morgan, 1994). Previously, this thermal profile has been related to ocular anatomy, principally the tear film being differentially warmed by a more exposed, thinner, avascular cornea surrounded by a highly vascularised, thicker limbus (Chapter 1.4). However, the dynamic OST changes observed and analysed in this study, such as the increase in thermal gradients as the eye is held open, suggest a more complex model for OST that also includes tear flow dynamics.

After a blink, a fluid layer of constant, uniform thickness would lose heat via convection and radiation, and any thermal profile would be solely due to the underlying structures. But the dynamic nature of the tear film as it thins and disperses further contributes to this model of thermo-dynamics. The tear film takes approximately 3 to 10 seconds to reach the most regular state following a blink (Nemeth et al., 2002), and the average thickness of the tear film has been shown to vary from approximately 9µm immediately after a blink to around 4µm just before the next blink (Mishima et al., 1966; Holly, 1981). The thermal effect of tear thinning will be to permit the ocular surface to contribute more to the measured radiation. Hamano's work would suggest that a tear film thickness of 4µm will absorb approximately 40% of radiation from the relatively cooler cornea (Hamano et al., 1969).

Just prior to blinking, the tear meniscus forms along the lower and upper lids: 70-90% of tear volume is found between these marginal tear strips (Mishima and Maurice, 1961). Studies on tear dynamics suggest that the wiper action of a blink brings fresh tears rapidly up over the ocular surface (Owens and Phillips, 2001), and these very quickly disperse towards the canthi, moving both temporally and nasally along the upper lid margin but only nasally along the lower lid margin (Fatt, 1992). The temporal end of the palpebral aperture closes more rapidly during a blink, further contributing to this dominant nasal passage of tears (Milder, 1965); along with some minimal effect of gravity (the surface tension of tears is relatively able to resist gravity in order for the tear film to exist in a vertical plane (Doane, 1984).

The decrease in OST following a blink was significantly different between areas, with the central area demonstrating the most significant change, and the inferior area of the ocular surface experiencing least change. The decrease was not necessarily greatest for the warmest areas of the ocular surface. Using accepted ideas about tear dynamics and drainage (Doane, 1981), it is proposed here that the tear meniscus at the lower lid will gather tear fluid via natural drainage processes, and hence, may be less susceptible to temperature change, whereas the central area will experience rapid tear-thinning over the same time period, resulting in greater temperature changes. It appears to be the movement of tears, not their initial temperature which determines the magnitude and rate of change in OST post-blink. The rates of cooling observed in this study generally agree with previous estimates of -0.030°C sec^{-1} (Morgan et al., 1996), and -0.033°C sec^{-1} (Efron et al., 1989).

4.4.3 Gender differences

The results concerning gender agree with other studies that have shown no apparent relationship between absolute values for OST and gender (Schwartz et al., 1968; Horven, 1975; Alio and Padron, 1982b; Morgan et al., 1993), Gender differences in dynamic OST are significant: the larger, and faster decrease in OST post-blink observed in females may be related to tear film properties: females have been shown to have a higher tear evaporation rate than males (Tomlinson and Giesbrecht, 1993); young females have been shown to have a higher basal tear flow rate (Henderson and Prough, 1950), and females generally show reduced tear film stability (Craig and Tomlinson, 1998). In addition, the corneal curvature of females has been found to be significantly steeper than that of males (Eysteinsson et al., 2002).

4.5 Conclusions

This large study offered unique insights into the thermal dynamics across the ocular surface following a blink. A complex model of thermo-dynamics is emerging:

- A film of fresh, warm tears from the lacrimal gland covers the ocular surface immediately after a blink.
- As soon as the eye is open, heat loss occurs from the tear film to the environment, via conduction, radiation and evaporation (Newton's Law of Cooling (Serway and Beichner, 2000).
- As the eye is held open, the drainage pattern of tears will encourage the warm tears to accumulate nasally, away from the central and temporal areas.
- As the eye continues to remain open, the tear film thins and evaporates from the more exposed areas first, allowing the underlying structures to contribute to the measured temperature profile.

To summarise, the thermal profile that is observed reflects the natural tear film dynamics within the palpebral aperture, and as the tear film thins the underlying ocular anatomy further enhances this temperature profile. However, as tear film thickness is still under debate (Korb et al., 2002), the exact contributions of each of these factors to the model during the post-blink period will remain indefinite.

CHAPTER 5

THERMAL PROPERTIES OF DIFFERENT CONTACT LENS MATERIALS MEASURED BY INFRARED THERMOGRAPHY

5.1 Introduction

The thermal properties of the eye have been modelled previously (Scott, 1988; van den Berg and Spekreijse, 1997), but the literature specific to similar modelling/experimental work with contact lenses *in vivo* is limited (Table 5.1).

MEDIUM	DENSITY (g/cm^3)	THERMAL CONDUCTIVITY (cal/cm.sec.°C)	SOURCE	DETAILS
bovine cornea	1.11	6.58×10^{-4}	Hamano et al, 1972	Part theory, part experimental
soft contact lens	1.18	6.64×10^{-4}	Hamano et al, 1972	Part theory, part experimental
all contact lens polymers	1.21	4.01×10^{-4}	Martin & Fatt, 1986	Heat transfer model
hard contact lens	1.04	4.4×10^{-4}	Hamano et al, 1972	Heat transfer model
human cornea	1.05	5.8×10^{-4}	Scott, 1988	Heat transfer model
water	1	1.48×10^{-3}	Martin and Fatt, 1986	Heat transfer model

Table 5.1
Physical constants of cornea and contact lenses taken from the literature.

Hamano and colleagues applied heat transfer modelling to bovine cornea and soft and hard contact lens materials (Hamano et al., 1972). In their study, the thermal conductivity of bovine cornea appeared very similar to that of soft contact lens material, but less than that of water, and hard contact lenses had a much lower thermal conductivity. A study using thermistors embedded in and sandwiched between contact lenses (Martin and Fatt, 1986) confirmed theoretical models by demonstrating a small flow of heat from the eye to the environment (1.1 x $^{10-2}$ cal.$^{cm-}$2.$^{sec-1)}$, which was similar to that from the rabbit eye (Rosenbluth and Fatt, 1977).

The thermal conductivity of contact lenses is traditionally calculated by considering the average density of the lens polymer and using an established relationship between polymer density and thermal conductivity. The total conductivity of a hydrogel lens is calculated by summing the weight fractions of polymer and water contained in the hydrated contact lens. In Martin & Fatt's 1986 study, contact lenses of higher water content appeared to cause a smaller rise in measured ocular temperature (behind the lens) than lenses of lower water content, and increasing lens thickness induced greater rises in measured temperature behind the lens for all levels of water content. The authors felt that these increases in OST (ranging from 0.5-1.5°C) were too small to be clinically significant in comparison to the potential influence of environmental conditions.

The disadvantages of contact methods of heat measurement have been discussed (Chapter 1.3.1): this study was designed to examine the insulating properties of popular hydrogel contact lenses using non-contact thermography as the method of temperature measurement. This was a two-part study:

Part A - Observation of the temperature effects of hydrogel contact lenses on a model eye.

Part B - Comparing model eye results to the observation of the temperature effects of hydrogel contact lenses *in vivo*.

5.2 PART A - MODEL EYE

5.2.1 Materials and Methods

A model eye was constructed from six light emitting diodes (LEDs) set into a resin dome (estimated radius of curvature 7.80mm), with a constant low voltage applied to produce a small amount of visible light and a level of thermal radiation similar to that produced by the human eye (31-36ºC). The radiated temperature was monitored using the Thermo Tracer TH7102MX thermo-camera (described in Chapter 2.1), positioned 60mm from the apex of the model eye. Temperature was collected from nine points across the contact lens area, and then averaged. The study was conducted within a controlled environment (room temp 21.4±0.5ºC and humidity at 39±1%), and the model eye was placed within a shallow chamber to protect it from draughts and reflected temperature changes (Figure 5.1).

All data was collected during a single session; the model eye was wiped dry and its temperature permitted to stabilise before the next contact lens was applied.

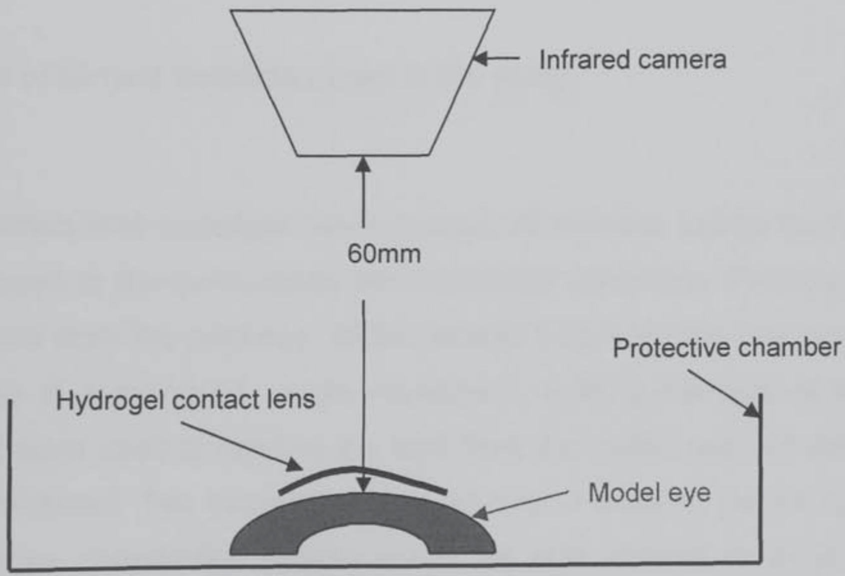


Figure 5.1
Experiment set-up using the model eye

Three types of hydrogel contact lenses were used in this study – balafilcon A, etafilcon A and lotrafilcon A (Table 5.2), and the experiment was repeated five times for each lens type (using different lenses each time, from the same batch). The same power (-3.00D) was selected for each lens type to keep any lens thickness variations solely a result of the lens design, rather than due to prescription.

MATERIAL	*ETAFILCON A*	*BALAFILCON A*	*LOTRAFILCON A*
Water content	58%	36%	24%
Base curve	8.5mm	8.6mm equivalent	8.6mm
Diameter	14.2mm	14mm	13.8mm
Wear/ Replacement schedule	Daily/ single use	Daily or continuous/ monthly replacement	Daily or continuous/ monthly replacement
Centre thickness (-3.00D)	0.084mm	0.09mm	0.08mm
Oxygen permeability (Dk, Fatt units)	28	99	140
Oxygen transmissibility (Dk/t, -3.00D)	33	110	175

Table 5.2
Specifications of contact lens types used in the study

The contact lens packages were opened 30 minutes before each experimental run, and exposed to the surrounding environmental conditions. Forceps were used to remove the lens from the package, shake excess fluid from the lens and place it onto the model eye in a practised, single movement, holding the lens at the edge. The same forceps were used to remove the lens from the model eye immediately after 10 minutes had elapsed. Ten minutes was chosen as a suitable period for observation: beyond this time dehydration effects made the lens difficult to remove. Time was monitored with a stopwatch. Temperature of the model eye was recorded dynamically from lens application to lens removal (inclusive), and the changes were normalised to baseline. Analysis of variance (repeated measures) was conducted to explore both the effect of time and contact lens type on model eye temperature. Post-hoc comparisons were employed to analyse significant time/group differences.

5.2.2 Results

Applying any contact lens to the model eye caused an immediate significant decrease in radiated temperature (figure 5.2): at 3 seconds after the contact lenses were applied the mean decrease was -4.63±0.1°C (n=15, p<0.0001).

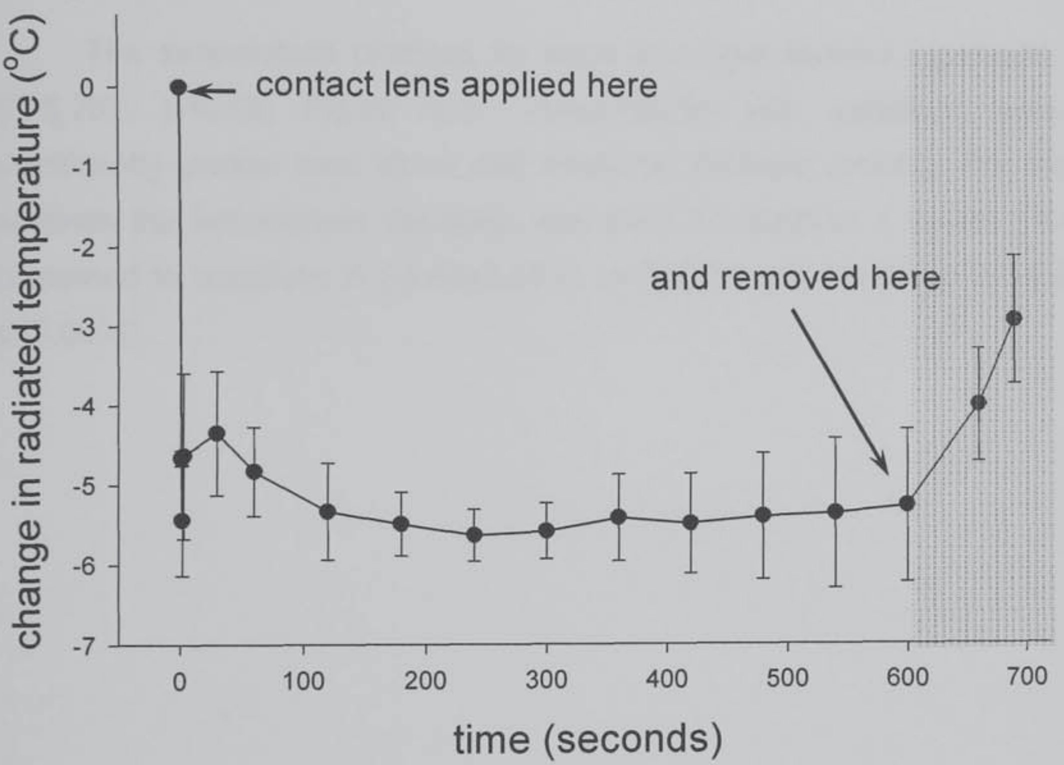


Figure 5.2
Mean temperature changes during 10 minutes of hydrogel contact lens 'wear' (n=15; error bars=1 SD)

Figure 5.2 shows the mean temperature changes observed when the contact lenses (n=15) were applied and removed after 10 minutes (all normalised to their respective baselines).

Time was a significant factor overall in radiated temperature from the model eye *during* lens application ($F=10.65$, $n=15$, $p<0.0001$). Post-hoc analysis (using Student Newman-Keuls test) revealed the significant changes in radiated temperature: a minimum temperature was observed at four minutes (-5.63±0.4°C; $p<0.05$ compared to the initial decrease); the mean temperature at ten minutes (just before the lenses were removed) had significantly increased compared to that at one minute ($p<0.05$). When contact lenses were removed mean temperature increased significantly (+1.28°C; $p<0.005$ compared to temperature at ten minutes). Radiated temperature then continued to increase towards baseline at a rate of 1.72°C.min^{-1}.

The temperature changes for each lens type showed significant differences ($F=5.707$; $p<0.05$; Figure 5.3). Temperatures with lotrafilcon were generally significantly greater than those with balafilcon (Scheffe; $p<0.05$). For the first three seconds the temperature decrease was least for etafilcon A lenses (-3.50±0.33°C) compared to balafilcon A (-5.41±0.38°C; $p<0.0001$) and lotrafilcon A (-5.03±0.45°C); $p<0.0001$).

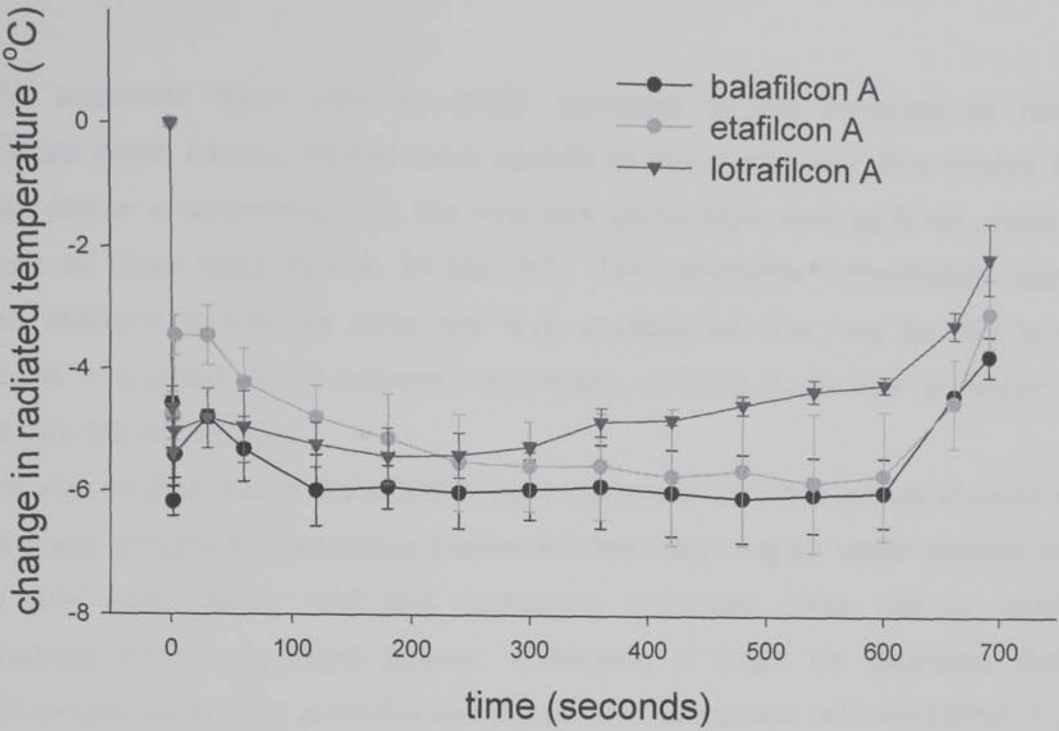


Figure 5.3
Mean (n=5) changes in temperature with three different hydrogel contact lenses *(contact lenses applied at 0, and removed at 600s; error bars=1 SD).*

Further temperature variations between lens types were insignificant, until eight minutes. At this point (and up to lens removal), radiated temperatures with lotrafilcon A lenses in situ were significantly greater than those with balafilcon A ($p<0.05$) and etafilcon A ($p<0.05$).

When the contact lenses were removed, a similar increase (average +1.08±0.3°C) in temperature was observed for all three lens types ($p=0.38$). The lotrafilcon A group remained significantly warmer than the other two for the first minute post lens removal ($p<0.005$).

5.2.3 Discussion

As expected there was an initial decrease in the measure of radiated temperature when contact lenses were applied to the model eye: this relates to the relatively cooler environment that the lens has come from (the buffered saline at a maximum of room temperature; 21.4±0.9°C). The difference immediately apparent between etafilcon A and the other two was unexpected and may be due to slight differences in sagittal depth between lens types, leading to greater or lesser initial contact with the model eye.

In vivo studies (using thermistors) have demonstrated that lenses of lower water content show greater temperatures behind the lens than higher water content lenses (Martin and Fatt, 1986), and that increasing thickness gives rise to increased temperatures behind hydrogel lenses. Therefore, it might be expected that the radiated temperature from a model eye would remain coolest with lotrafilcon A (24% H_2O; thinnest lens type). However, these results appear to suggest the opposite: that the lotrafilcon A lens 'warms up' during the observation period whereas the other lens materials do not to the same extent. There are important distinctions here that help explain the 'disparity':

a) For lens thicknesses around 0.08mm and water contents from 20-60%, the data from Martin & Fatt suggests only an effect of ±0.05°C.

b) Studies using thermistors behind a contact lens say little about the heat flux to the atmosphere through the lens over time.

c) In model eye studies like this one, dehydration and evaporation begins as soon as the lens is removed from its packaging, and there is no blink effect to counter this.

Physical laws, such as Newton's Law of Cooling, dictate that heat will be transferred from the warmer medium to the cooler one and the rate of such transfer is dependent on the physical properties of such environments, including the temperature difference between them (Serway and Beichner, 2000). It is suggested, therefore, that the temperature build-up behind any lens gives rise to a temperature gradient, which in turn encourages conduction of such heat through the lens material towards the thermal camera. The relative differences in dehydration and evaporation between the different lens types will affect this rate of heat transfer. Greater lens water content may mean that radiated surface temperature from the contact lens remains cooler for longer than lower water content.

5.2.4 Conclusion

The thermal properties of a contact lens material will affect ocular surface temperature (OST) when hydrogel contact lenses are worn and this effect differs over time.

5.3 PART B - HUMAN EYE

The usefulness of the model eye was found to be limited to ten minutes due to dehydration effects, so the next stage was to look at similar scenarios in the human eye. This study was designed to observe the temperature effects of inserting contact lenses and the first two hours of contact lens wear.

5.3.1 Materials and Methods

The right eyes of 12 young volunteers were used in this study (mean age 24.6±3.2yrs; 6m, 6f). Inclusion criteria specified that all subjects had:

a) no history of eye disease
b) not habitual contact lens wearers
c) good general health and well-being
d) no dry eye symptoms (itchiness, grittiness, etc)

Subjects attended for two, mid-afternoon sessions. Prior to the experiment, each subject was asked about their general health and examined for signs of ocular inflammation using a slit-lamp, and an overlay template was constructed for each subject to aid alignment with the thermal camera during repeated observations (Chapter 2.1.3). This preparation period allowed for suitable adaptation to room temperature in the laboratory (21.4±0.9°C; 40% humidity), which has been shown previously in this thesis to be 10 minutes for subjects coming from within the same building (Chapter 2.2.1). Data collection and analysis was as described in Chapter 2.1.4. The contact lens packages (same batch; all -3.00DS) were opened 30 minutes before each experimental run, and exposed to the surrounding environmental conditions. A preliminary measure of OST (average of 3 repeats) for each subject was made at each session, before a contact lens was inserted by the researcher: subjects were randomly assigned either a lotrafilcon A or balafilcon A contact lens (lens properties shown in Table 5.3), so that they had worn both lens types by the end of the second session. Radiated temperature from the central area (which covers the main body of the contact lens) was recorded, as described in Chapter 2.1. Measurements were repeated at 2, 10, 20, 30, 60 and 120 minutes after lens insertion. After 120 minutes, the contact lens was removed and an immediate measure taken of OST. A final measure of OST was taken 5 minutes after lens had been removed. Results for each subject were normalised to baseline (the preliminary OST), and changes with time averaged for both lens types. Repeated measures analysis of variance was used to compare the within-group differences (time) and between-group differences (lens type).

MATERIAL	***LOTRAFILCON A***	***BALAFILCON A***
Water content	24%	36%
Base curve	8.6mm	8.6mm equivalent
Diameter	13.8mm	14mm
Power	-3.00	-3.00
Wear/ Replacement schedule	Daily or continuous/ monthly replacement	Daily or continuous/ monthly replacement
Centre thickness (-3.00D)	0.08mm	0.09mm
Oxygen permeability (Dk, Fatt units)	140	99
Oxygen transmissibility (Dk/t, -3.00D)	175	110

Table 5.3
Specifications of silicone hydrogel contact lenses used in the study (part B)

5.3.2 Results

The temperatures (normalised) presented here relate to the average temperature of nine points in the central area, immediately (0.2s) after a blink.

5.3.2.1 Change in OST with contact lens wear

Radiated temperature decreased when hydrogel lenses were inserted for both lens types, in all subjects (average decrease -0.65±0.22°C). Mean temperature (all lenses, n=24) continued to decline in for at least 30 minutes; after one hour temperature increased towards baseline. When contact lenses were removed, measured OST was greater: for 80% of cases OST was above baseline measures (Figure 5.4). As contact lenses were worn, the main effect of time was statistically significant ($F=65.99$, $p<0.0001$).

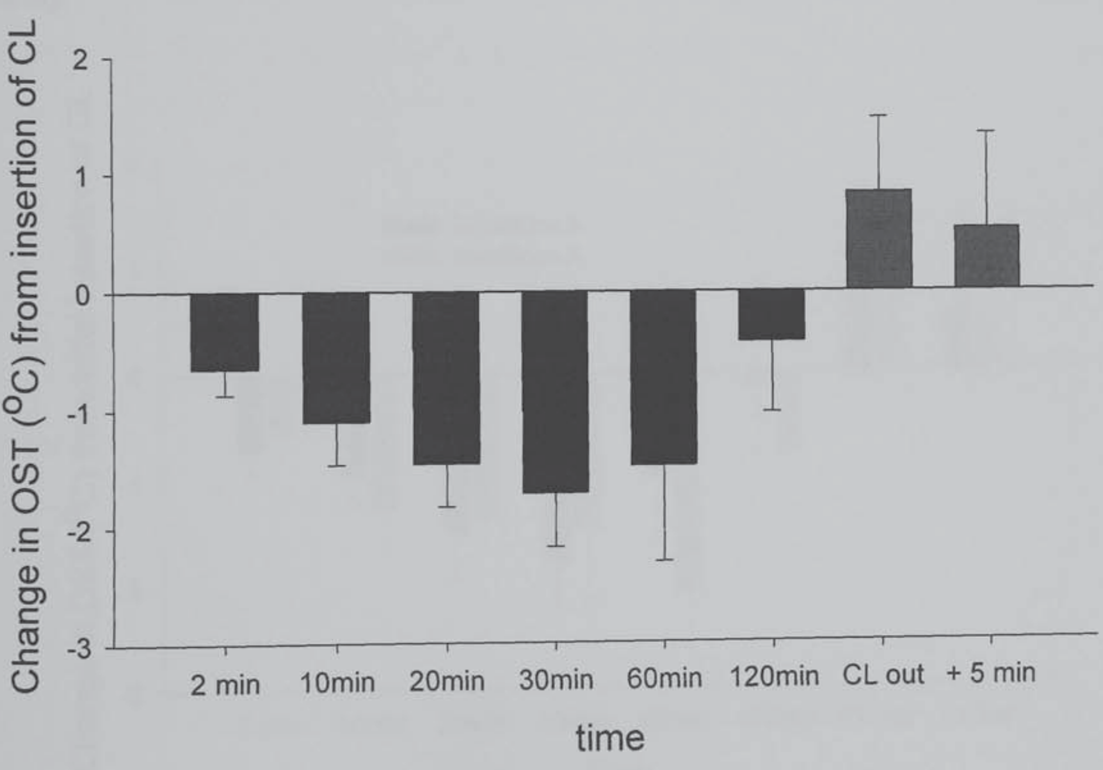


Figure 5.4
Change in OST over two hours of silicone hydrogel contact lens wear (lotrafilcon A & balafilcon A; n=24, error bars =1sd)

Post-hoc comparisons revealed a further significant decrease in average OST between 2 and 20 minutes (Scheffe; $p<0.005$), followed by a significant increase between 30 and 120 minutes (Scheffe; $p<0.0001$). When contact lenses were removed OST significantly increased (average increase +0.84±0.65°C above baseline; Scheffe; $p<0.0001$), but further change was insignificant.

5.3.2.1 Effect of lens type on OST

The average results for each lens type with time are shown in Figure 5.5. The effect of CL type (balafilcon A vs. lotrafilcon A) on mean temperatures over the whole experiment was insignificant ($F=3.08$, $p=0.10$, power 36%); however, the effect of CL type on temperature with time (i.e. interaction effect) was statistically significant ($F=8.22$, $p<0.0001$). When the results from one hour and onwards were analysed separately, the effect of contact lens type was significant ($F=5.52$, $p<0.05$, power 58%).

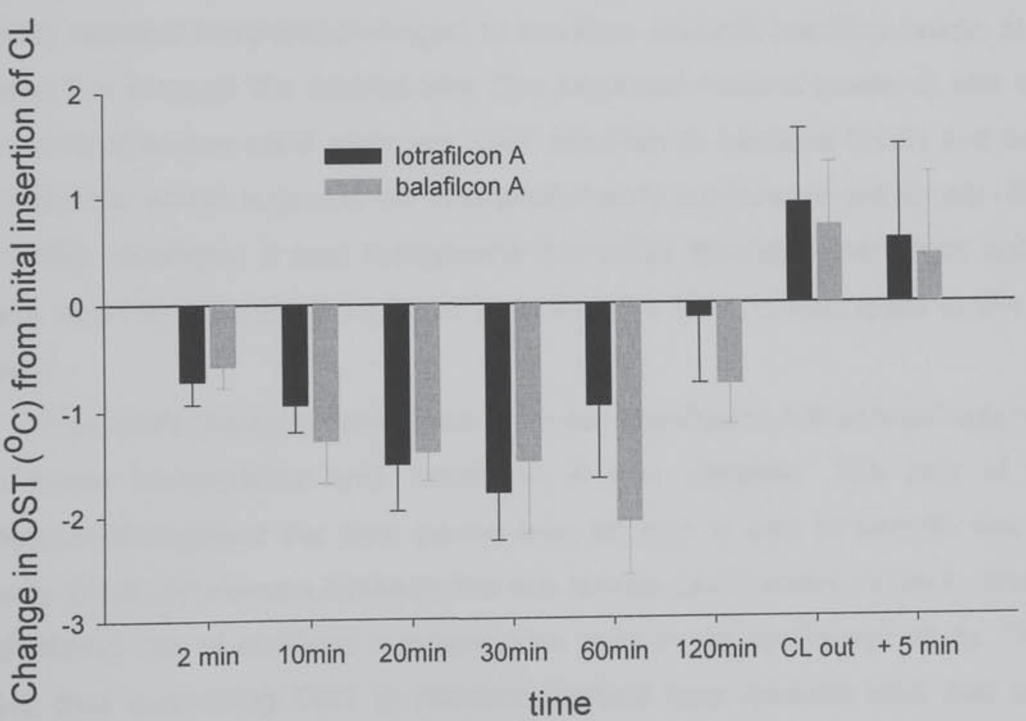


Figure 5.5
Change in OST over two hours of hydrogel contact lens wear (n=8 for each lens type; error bars=1sd)

5.3.3 Discussion

The observed initial decrease in temperature when contact lenses are inserted mimics that seen on the model eye in part A, but is of much less magnitude (-0.65±0.22°C cf. -4.63±0.1°C). This is not surprising given the rapid warming effect of fresh tears from the lacrimal gland: there will have been some initial reflex tearing upon lens insertion in these neophyte subjects. The tear film on top of contact lenses is less stable and thinner (Guillon et al., 1997), and destabilizes further after a blink (Korb, 1994), which means that the contact lens surface temperature will be the major influence on the actual measured temperature (after Hamano et al., 1969). Evidence of an insulating effect due to the contact lenses was evident: tear exchange with hydrogel contact lenses is only about 10-20% (Hayashi and Fatt, 1976), and despite the potential warming effect that each blink produced, recorded temperature continued to decrease after insertion for at least 30 minutes in all subjects. This continued decrease may be due to evaporation from the anterior lens surface, similar to the model eye. After a variable period of time (between 30 and 60 minutes for most subjects) radiated temperature began to increase towards baseline levels, suggesting that heat flux through the contact lens (the expected thermal gradient) was occurring. When contact lenses were removed, OST returned to baseline levels and beyond for most subjects, which supports the insulation theory put forward previously (Martin and Fatt, 1986). However, it was recognised that reflex tearing in neophyte contact lens wearers upon lens removal could be a factor (this topic is discussed in the following chapter).

Differences between lens types were not significant until at least one hour, after which mean temperature with lotrafilcon A was greatest. The lack of statistical significance throughout the time period may be due in part to sample size, and the relatively small differences between the two lenses used (water content), compared to the additional use of etafilcon A lenses also used in the model eye study. The results suggest that examining OST in habitual contact lens wearers who had worn their lenses at least two hours might reveal more significant (both statistically and clinically) differences between lens types (Chapter 6).

5.4 Conclusions

An extension to previous models of heat transfer with contact lenses in situ is proposed (Figure 5.6).

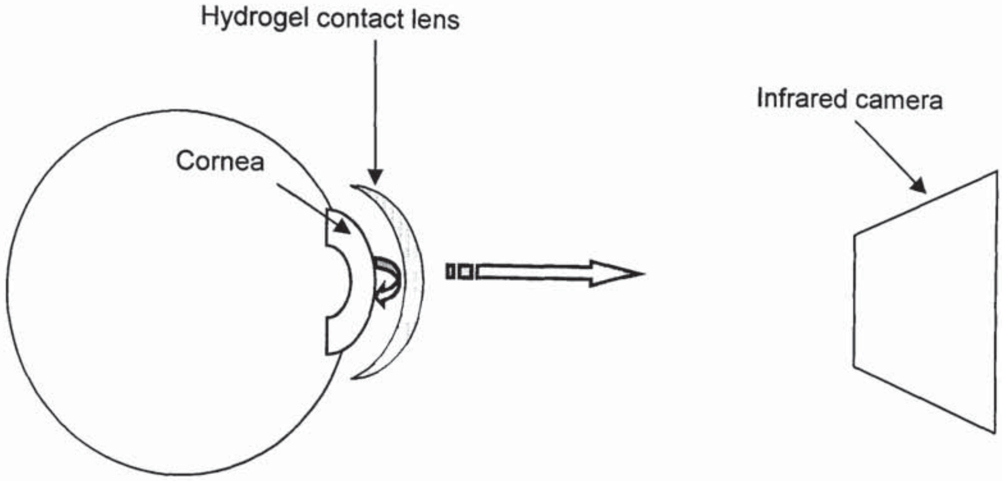


Figure 5.6
Schematic diagram to demonstrate heat transfer with hydrogel contact lenses

Applying a new hydrogel contact lens to the eye causes a decrease in radiated temperature, modified by initial reflex tearing. The measured temperature is likely to (at least initially) remain below normal OST as it is probably the temperature of the contact lens surface (the pre-lens tear film is thin); until such point that the lens surface is warmed by the natural heat flux from the anterior eye. The rate at which this heat transfer occurs is likely to depend on several factors:

- lens water content
- lens thickness
- lens fit
- environmental conditions
- how long the lens has been worn
- neophyte/ adapted lens wearer

Because the thermal conductivity of contact lenses will always be less than the tear film and cornea (due to differences in water content), it might be anticipated that a temperature gradient remains between the post-lens tear film and the anterior surface of the contact lens throughout wear. Observed increases in radiated temperature upon lens removal appear to support this theory.

Chapter 6 examines experienced hydrogel contact lens wearers to observe temperature with lenses in situ and just after removal, with lenses having been worn for at least two hours.

CHAPTER 6

THE EFFECTS OF CONTACT LENS WEAR ON OST

6.1 Introduction

Contact lens wearers often use the terms 'hot' or 'burning' to describe their symptoms, and 'coolness' can be associated with relief from discomfort (Du Toit et al., 2001). This is particularly the case in patients with dry eye symptoms (McMonnies, 1986; Bandeen-Roche et al., 1997; Begley et al., 2002). Conjunctival hyperaemia has been suggested to be a measure of the ocular response to contact lens wear, whether symptomatic or otherwise (McMonnies et al., 1982; Bron et al., 1985; McMonnies and Chapman-Davies, 1987; Holden, 1989), and changes in blood flow to the sclera or conjunctiva have been shown to produce changes in local temperature (Mapstone, 1968b). Grading this hyperaemic response has attracted much interest from researchers and clinicians alike (Efron, 1998; Wolffsohn and Purslow, 2003).

There are, however, many occasions when the ocular response to contact lens wear may be initially minimal, particularly with the advent of silicone hydrogel contact lenses (Dumbleton, 2002), and alternative non-contact methods to conventional slit lamp examination will be required to observe the ocular response.

There have been few reports in the literature observing temperature of the anterior eye during contact lens wear: most of these studies have used contact methods of temperature measurement (Table 6.1).

DATE	REFERENCE	SUBJECT NUMBERS	METHOD	RESULTS
1965	Hill and Leighton	8 eyes	• thermistors under scleral contact lenses • studied effect of eye closure and palpebral aperture	• eye slightly cooler with scleral lens in place, but not significant (31.3°C • eye closure produced significant increase in temperature (33.3°C) • temperature stabilises after 4 minutes • vertical apertures <6mm produced significant increases in temperature
1969	Hamano *et al*	10 eyes	• use of a non-contact radiometer and rigid contact lenses	• less than 0.5°C difference was observed between eyes with and without rigid contact lenses • open eye-32.0°C; closed eye-34.2°C
1980	Fatt & Chaston	6 eyes	• measured temperature with and without hard and soft contact lenses • use of a non-contact bolometer • use of a thermistor in fornix under closed eye conditions	• temperature wearing hard contact lenses was 0.5-1.5°C lower than that of bare cornea • temperature wearing soft contact lenses was never more than 0.5°C lower than that of bare cornea • observed increased temperature under closed eye conditions, especially when a contact lens is worn
1986	Martin & Fatt	13 eyes	• describes modelling and experiments to investigate the surface temperature beneath hydrogel contact lenses • thermistors sandwiched between thin hydrogel contact lenses	• demonstrated small flow of heat from eye to environment • insignificant increases in anterior corneal surface temperature beneath a contact lens predicted by model • increase in temperature observed under closed eye conditions (34.5°C compared to 36.2°C)
1991	Montoro *et al*	38 subjects, including 19 contact lens wearers	• observations of the effects of eye rubbing and contact lens wear • use of a scanning infrared camera (true thermography) and customised software for objective analysis of the digital thermal image	• observed that some subjects with increased lacrimation showed a significant cooling in temperature • observed a minimal, transient effect of eye rubbing, concurrent with a small effect on fellow eye • observed an irregular thermal pattern in contact lens wearers

Table 6.1
Previous studies examining the effect of contact lenses on eye temperature

It is difficult to compare such studies as it has been shown that eye temperature is influenced by many factors including environmental conditions (Schwartz, 1965; Freeman and Fatt, 1973; Rysa and Sarvaranta, 1974), lid position (Hill and Leighton, 1965b), ocular health (Mapstone, 1968d; Mikesell, 1978; Morgan, 1994), age (Horven, 1975; Alio and Padron, 1982b; Girardin et al., 1999; Morgan et al., 1999) and point of measurement on the cornea (Fielder et al., 1981; Alio and Padron, 1982a; Efron et al., 1989): as discussed in Chapter 1.5. These factors have varied between studies, in addition to different measurement techniques.

The literature and the results from Chapter 5 suggest that OST will be affected by contact lens wear, particularly in a dynamic way: there are short term changes in the tear film when a contact lens is inserted: a contact lens disrupts the integrity of the lipid layer and increases tear film evaporation (Korb et al., 2002). The physical properties of the tear film above and beneath the contact lens are likely to influence OST. For instance, volume and mixing of the post-lens tear film (PoLTF) depends on factors such as blinking, lens edge profile, tear production and drainage and lens material (Lin et al., 2003). For the pre-lens tear film (PreLTF), a thin lipid layer will form over a hydrogel lens at best, but with a rigid lens that moves more, the lipid layer will be absent (Korb, 1994). With the resurgence in popularity of continuous wear with silicone hydrogel lens materials, the effect of eye closure on ocular physiology has received renewed attention (Ladage et al., 2003; Nichols and King-Smith, 2003). Previous eye temperature studies have recognised that closing the eyelids exposes the anterior eye to the warming effect of the tarsal conjunctiva and fresh tears from the lacrimal gland (Hill and Leighton, 1965b; Mapstone, 1968b), and eye closure has been observed to result in 1.5°C rise in OST (Mapstone, 1968b; Holden and Sweeney, 1985). No work has been published concerning the effect on OST of closed eye conditions during continuous contact lens wear. OST may be important to the sub-acute state of inflammation that is found in closed-eye environment (Sack et al., 2003): it is generally recognised that eye closure leads to significant changes in the tear film which allow the proliferation or accumulation of bacteria (Sack et al., 1992;

Sack et al., 2003), and that contact lens wear modifies the ocular bacteria found (Stapleton et al., 1995; Ren et al., 2002).

The purpose of this study was to examine the influence of hydrogel and silicone hydrogel contact lens wear on ocular surface temperature (OST), in experienced wearers, to include the dynamic changes in OST after a blink for different lens materials and modalities of wear.

6.2 Method

6.2.1 Study population

GROUP	*CONTACT LENS & WEAR MODALITY*	*NUMBER*	*GENDER*		*MEAN AGE ± SD (YEARS)*	*AGE RANGE (YEARS)*	*RANGE OF REFRACTIVE ERROR (MSE)*
Control	None	8	3m	5f	23.5 ± 2.8	19-28	+0.75 to -7.50
LDW	lotrafilcon A - *daily wear*	8	2m	6f	20.8 ± 1.5	18-24	+1.50 to -7.00
LCW	lotrafilcon A - *continuous wear*	8	6m	2f	20.6 ± 1.9	18-24	+1.75 to -7.00
BDW	balafilcon A - *daily wear*	8	4m	4f	20.0 ± 1.7	18-23	-0.75 to -6.50
BCW	balafilcon A - *continuous wear*	8	2m	6f	20.0 ± 1.7	18-23	-0.50 to -5.00
EDW	etafilcon A - *daily wear*	8	4m	4f	22.6 ± 2.6	19-25	-0.50 to -6.00

Table 6.2
Details of subjects used in the study

The subject sample consisted of the right eyes of 48 young, healthy volunteers (Table 6.2); 21 males and 27 females. Eight control subjects had never worn any contact lenses; forty contact lens wearers had all worn soft lenses successfully, to the specified modality for at least one year prior to the start of the study, and had a well-fitting contact lens with no history of ocular inflammation. Subjects were examined on a slit lamp and were excluded if they showed any sign of ocular inflammation or general ill-health, or were taking any medication, at the time of presentation. Full ethical approval from the University Human Sciences Ethical Committee was granted prior to commencement of the study and informed consent was obtained from every subject. All procedures conformed to the tenets of the Declaration of Helsinki.

6.2.2 Study design and procedure

All subjects attended randomly from each group over the course of the study, mid-morning, between 10.00 am and 11.00am. Daily contact lens wearers were required to have been wearing their lenses at least two hours, and continuous wearers were asked to confirm that the lenses had been in continuously for at least two weeks. Temperature measurements took place within the controlled environment of the laboratory (described in Chapter 2.1), with room temperature maintained at 21.4±0.5°C, humidity at 39±1%. Subjects adapted to the room conditions for at least 10 minutes before measurements were taken (Chapter 2.2.1), and were asked to refrain from rubbing their eyes prior to and during the session. During the adaptation period, the thermo-camera was positioned with respect to lower lid and canthi for each subject, so that the geometric centre of cornea could be aligned with the system (Chapter 2.1.3).

For all subjects three repeated measurements were made of the dynamic surface temperature: subjects blinked normally and, when instructed, held their eye open for eight seconds. Contact lens wearers were then asked to remove their right contact lens as gently as possible, and then three further readings were taken, over a period of 1 minute, without the contact lens. To remove the contact lens, subjects were instructed to digitally decentre onto the sclera and then pinch the lens off the eye.

In order to evaluate any temperature effects that may result purely from physically manipulating a contact lens in this way, three randomly chosen subjects were asked to return for a second session during which OST was similarly recorded with lenses in situ and after digitally moving the lens to one side and allowing it to re-centre.

6.2.3 Data acquisition and analysis

OST was recorded from 23 points across the ocular surface and grouped into five areas (as described in Chapter 2.1.3). Initial OST following a blink and dynamic changes in OST over the eight-second post-blink period were analysed and compared using one-way ANOVA. Significant differences between groups were evaluated with Scheffe's post-hoc testing. Pearson's correlation coefficients were used to correlate OST with and without contact lenses. All analyses were performed using Excel, version XP (Microsoft Corporation, Redmond, USA) and StatView, version 5 (SAS Institute Inc., Cary, USA).

6.2.4 Contact lens types

The physical characteristics of the contact lenses used for this study are shown in Table 6.3.

MATERIAL	***Etafilcon A***	***Balafilcon A***	***Lotrafilcon A***
Water content	58%	36%	24%
Base curve	8.5mm, 9.0mm	8.6mm equivalent	8.4mm, 8.6mm
Diameter	14.2mm	14mm	13.8mm
Wear/ Replacement schedule	Daily/ single use	Daily or continuous/ monthly replacement	Daily or continuous/ monthly replacement
Centre thickness (-3.00D)	0.084mm	0.09mm	0.08mm
Oxygen permeability (Dk, Fatt units)	28	99	140
Oxygen transmissibility (Dk/t, -3.00D)	33	110	175

Table 6.3
Specifications of contact lens types used in the study

6.3 Results

Digitally manipulating a contact lens (n=3) was found to have an insignificant effect on the measured temperature (*average temperature change=0.18±0.1°C; p=0.28*).

For the readers' ease, the following results are grouped into temperatures with *lenses in situ*, and *with lenses removed*. For each section *initial OST post-blink* and *dynamic changes in OST* are presented.

6.3.1 Temperatures recorded with lenses in situ

6.3.1.1 Initial temperature immediately post-blink

Central OST for the control group was significantly less than the mean temperature recorded for all contact lens wearers (34.96±1.06°C vs. 36.43±1.59°C; p<0.05). This was true for all five areas of the ocular surface (Table 6.4).

GROUP	CENTRAL AREA	NASAL AREA	SUPERIOR AREA	INFERIOR AREA	TEMPORAL AREA
CONTROLS (n=8)	34.96±1.07°C	35.29±1.07°C	35.02±1.23°C	34.93±1.09°C	34.89±1.09°C
CONTACT LENS WEARERS (n=40)	36.43±1.59°c	36.97±1.63°C	36.85±1.56°C	36.39±1.67°C	36.35±1.58°C
STUDENT'S T-TEST to compare group means	*t=2.50; p<0.05*	*t=2.78; p<0.01*	*t=3.11; p<0.005*	*t=2.36; p<0.05*	*t=2.48; p<0.05*

Table 6.4
Mean values of OST (±1sd) for controls and surface temperature for contact lens wearers (*lenses in situ*)

Within the contact lens wearers, there were significant differences in surface temperature between the groups (one way ANOVA; F=7.05, p<0.0005 - central area). Scheffe post-hoc analysis revealed the most significant differences were between EDW and LDW (34.66±0.85°C vs. 36.68±1.41°C; p<0.05), EDW and LCW (34.66±0.85°C vs. 37.78±1.19°C; p<0.001), and between EDW and BDW (34.66±0.85°C vs. 37.01±1.25°C; p<0.05). Similar differences between lens types were found in all areas (Figure 6.1).

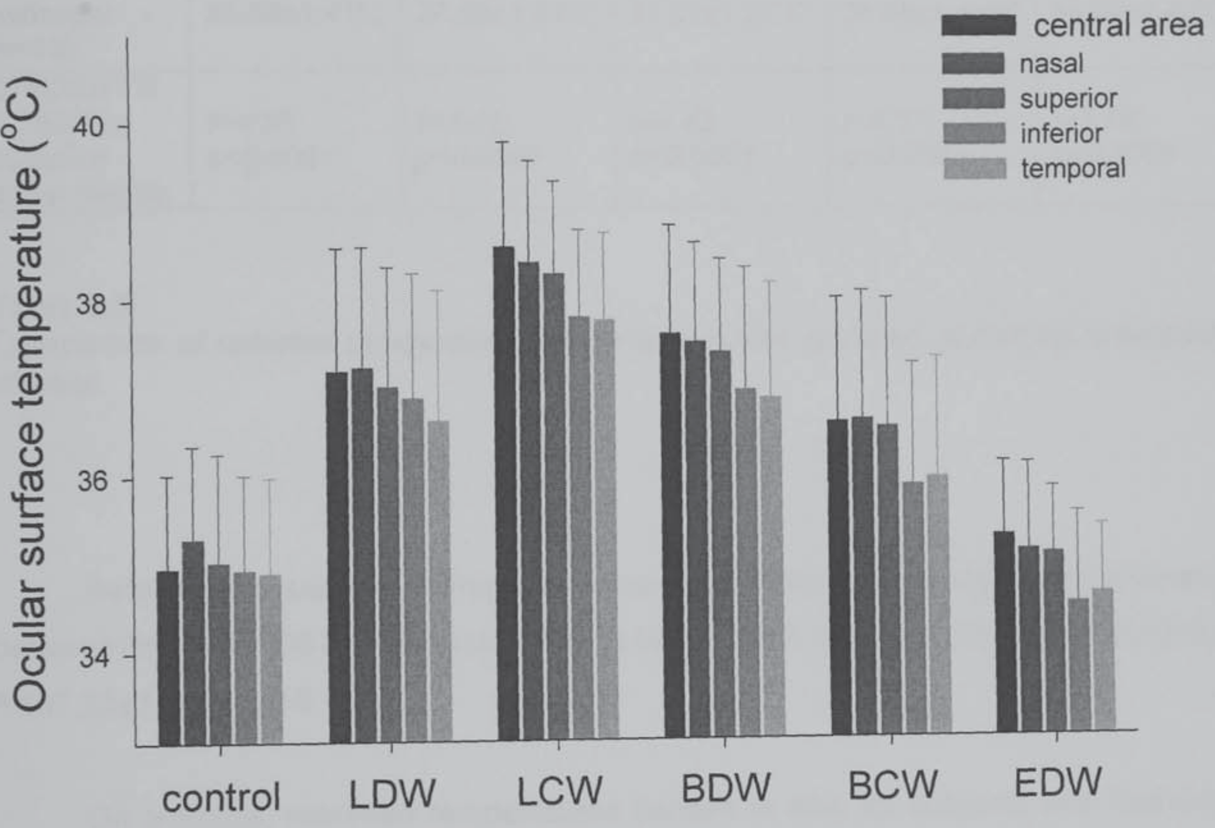


Figure 6.1
Initial OST of all groups (*lenses in situ*).

Significant differences in temperature were observed within the contact lens wearing groups according to *lens material*: subjects wearing etafilcon A exhibited lower temperatures than those wearing silicone hydrogel lenses (table 6.5).

GROUP	CENTRAL AREA	NASAL AREA	SUPERIOR AREA	INFERIOR AREA	TEMPORAL AREA
etafilcon A (n=8)	34.66±0.85°c	35.10±1.01°C	35.07±0.76°C	34.50±1.05°C	34.61±0.78°C
silicone hydrogel (n=32)	36.88±1.41°c	37.48±1.41°C	37.31±1.37°C	36.88±1.44°C	36.80±1.42°C
STUDENT'S T-TEST to compare group means	*t=4.25; p<0.0001*	*t=4.41; p<0.0001*	*t=4.42; p<0.0001*	*t=4.37; p<0.0001*	*t=4.18; p<0.0005*

Table 6.5
Comparison of radiated temperature with lenses in situ, grouped according to modality of wear.

Between the silicone hydrogel lens wearers, there was no significant difference between the initial OST of subjects wearing balafilcon A (36.51±1.38°C) and lotrafilcon A (37.23±1.38°C; p=0.16).

On average, recorded temperatures (lenses in situ) for subjects who had slept in their contact lenses (LCW, BCW; n=16) was greater than that of those who had inserted contact lenses that morning (LDW, BDW, EDW; n=24), but this difference only approached significance (Figure 6.2; 'p' values ranged from 0.06 to 0.17).

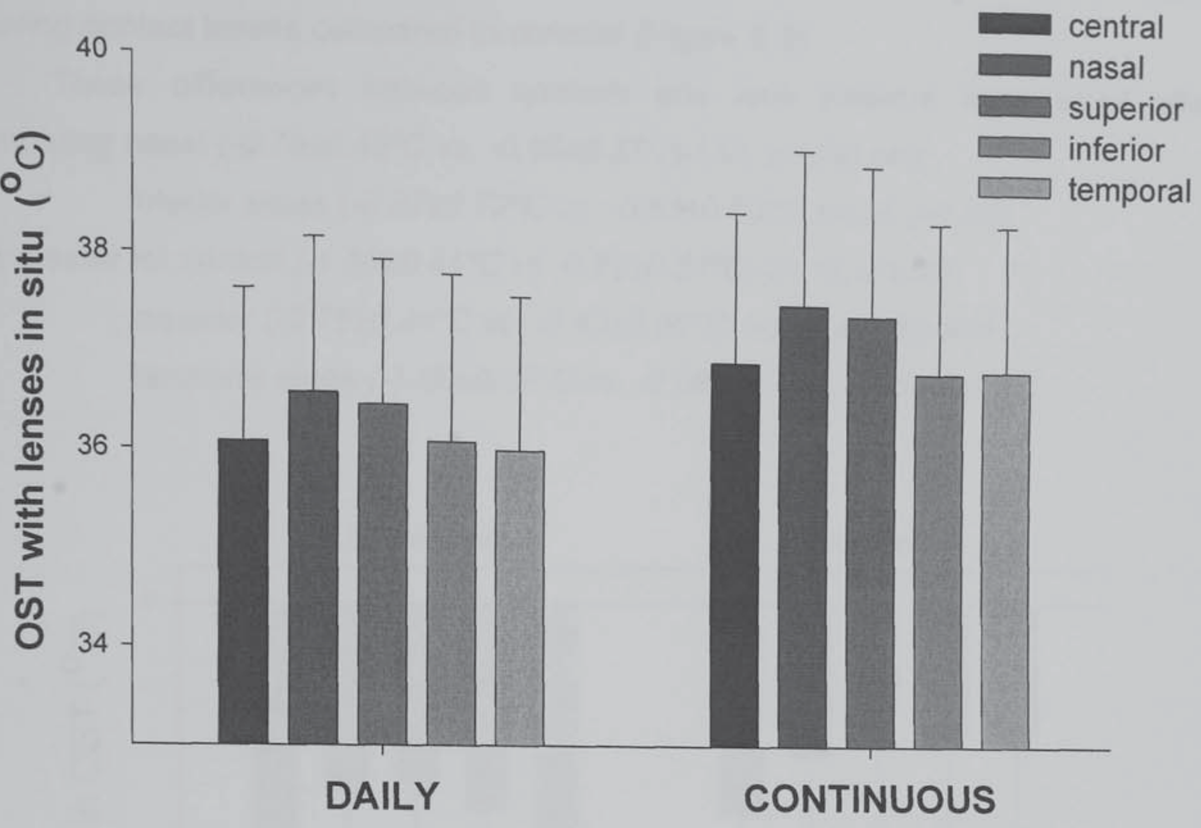


Figure 6.2
Radiated temperature with *lenses in situ*, comparing modality of wear (daily wear, n=24 vs. continuous wear, n=16)

Within the silicone hydrogel lens wearing groups, modality of wear appeared to have no effect on recorded temperatures with lenses in situ (central area: DW 36.83±1.30°C vs. CW 36.93±1.54°C; p=0.86).

6.3.1.2 Post-blink changes in OST, with contact lenses in situ

Dynamic changes in OST for eight seconds following a blink were analysed by examining at the *quantity* and the *rate of change*.

In the central area, *all* subjects showed an overall decrease in OST over eight seconds after a blink. At least 94% of subjects similarly showed a decrease in OST post-blink in all other areas.

The average decrease in OST in each of the five areas was *greater* for subjects wearing contact lenses compared to controls (Figure 6.3).

These differences between controls and lens wearers were small when comparing nasal (*-0.79±0.42°C vs. -0.65±0.37°*, t=0.87, p=0.39) and

inferior areas (*-0.87±0.72°C vs. -0.61±0.59°C*, t=0.96, p=0.35),

but greater for central (*-1.30±0.61°C vs. 0.90±0.51°C;* t=1.73; p=0.09),

superior (*-0.76±0.41°C vs. -0.43±0.50°C; t=2.00; p=0.05*) and

temporal areas (*-1.08±0.57°C vs. -0.58±0.47°C; t=2.31; p<0.05*).

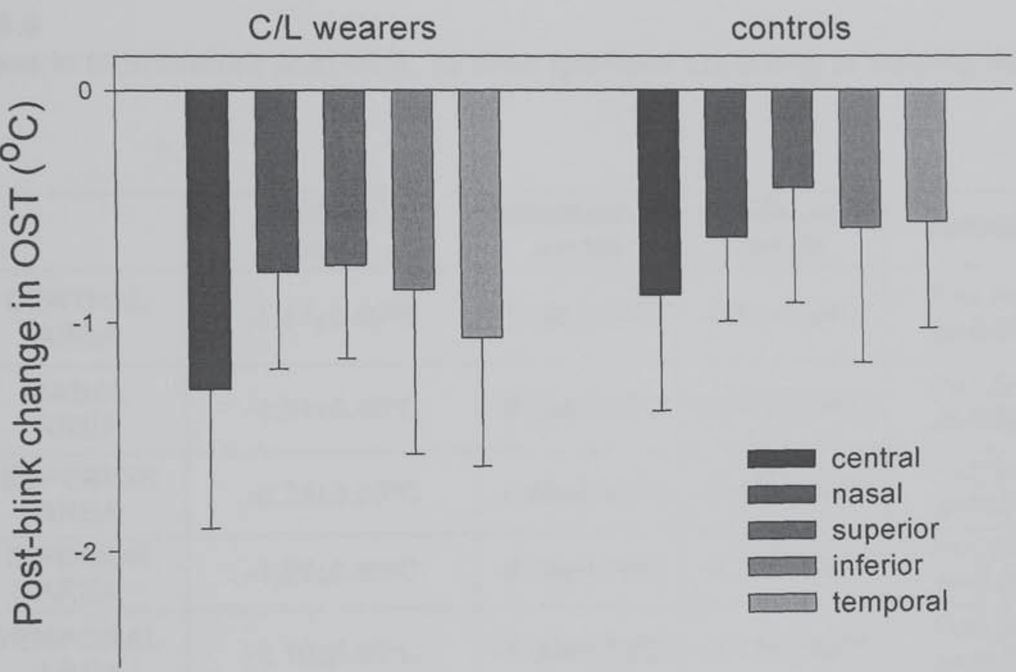


Figure 6.3
Decrease in OST over eight seconds following a blink, for controls (n=8) and subjects wearing contact lenses (n=40)

Within the contact lens-wearing groups there was no significant difference in the magnitude of post-blink change in OST for neither lens material nor modality. Similar results were obtained across all areas of the anterior eye (Tables 6.6, 6.7).

	DAILY WEAR (n=24)	CONTINUOUS WEAR (n=16)	*STUDENT'S T-TEST*
CENTRAL AREA	-1.28±0.67°c	-1.32±0.53°C	*t= -0.22;* ***p=0.83***
NASAL AREA	-0.78±0.49°c	-0.79±0.30°C	*t= -0.08;* ***p=0.94***
SUPERIOR AREA	-0.71±0.43°C	-0.83±0.37°C	*t= -0.85;* ***p=0.40***
INFERIOR AREA	-0.89±0.83°C	-0.83±0.54°C	*t= 0.26;* ***p=0.80***
TEMPORAL AREA	-1.03±0.61°C	-1.15±0.51°C	*t= -0.63;* ***p=0.53***

Table 6.6
Decrease in temperature post-blink, by area (grouped according to wearing modality)

	etafilcon A (n=8)	lotrafilcon A (n=16)	balafilcon A (n=16)	*ANOVA*
CENTRAL AREA	-1.47±0.60°C	-1.18±0.68°C	-1.33±0.54°C	*F=0.64;* ***p=0.53***
NASAL AREA	-1.01±0.40°C	-0.69±0.51°C	-0.77±0.30°C	*F=1.57;* ***p=0.22***
SUPERIOR AREA	-0.74±0.38°C	-0.68±0.46°C	-0.85±0.37°C	*F=0.64;* ***p=0.53***
INFERIOR AREA	-1.27±0.85°C	-0.76±0.79°C	-0.77±0.50°C	*F=1.66;* ***p=0.21***
TEMPORAL AREA	-1.10±0.57°C	-1.00±0.63°C	-1.14±0.53°C	*F=0.24;* ***p=0.79***

Table 6.7
Decrease in temperature post-blink, by area for contact lens groups (grouped according to lens material)

For the silicone hydrogel lens wearers, the magnitude of the post-blink change was greater for continuous wear compared to daily wear, across all areas, but this was not significant (central area 1.32±0.53°C vs. 1.17±0.71°C; t= -0.66; p=0.51).

With lenses in situ, the *rate of the cooling* following a blink was fastest in the contact lens wearing groups compared to controls, but these differences were only statistically significant in the temporal and superior areas (Figure 6.4, Table 5.8).

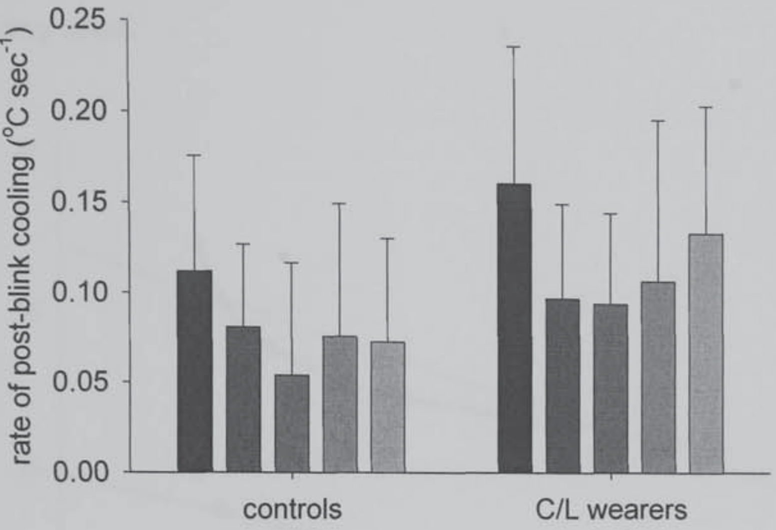


Figure 6.4
Average rate of post-blink cooling (°C/sec) for controls (n=8) and contact lens wearers (n=40), in five areas across the anterior eye

	CONTROLS (n=8)	WITH CONTACT LENSES (n=40)	*STUDENT'S T-TEST*
CENTRAL AREA	0.16 ±*0.08*	0.11 ±*0.06*	*t=1.73;* ***p=0.09***
NASAL AREA	0.10 ±*0.05*	0.08 ±*0.05*	*t=0.88;* ***p=0.39***
SUPERIOR AREA	0.10 ±*0.05*	0.05 ±*0.06*	*t=2.00;* ***p=0.05***
INFERIOR AREA	0.11 ±*0.09*	0.08 ±*0.07*	*t=0.96;* ***p=0.34***
TEMPORAL AREA	0.14 ±*0.07*	0.07 ±*0.06*	*t=2.31;* ***p<0.05***

Table 6.8
Average rate of cooling post-blink by area, for controls and contact lens wearers (°C sec^{-1} ± 1sd)

The times taken to reach ¼, ½ and ¾ of the post-blink temperature change were plotted for controls and contact lens wearers, for the central area (Figure 6.5). The data suggest a faster decline in temperature initially for contact lens wearers compared to controls, but differences for ¼, ½, and ¾ 'lives' were not significant statistically in any area.

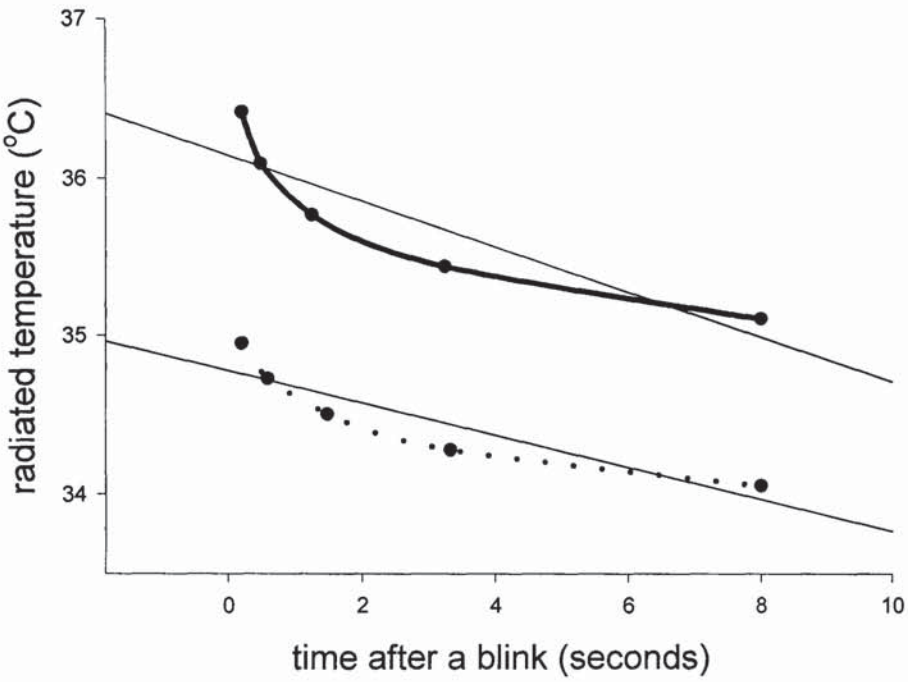


Figure 6.5
The post-blink change in temperature for controls (broken line) and contact lens wearers (solid line) over 8 seconds (central area)

Within the contact lens wearing groups, there was no significant difference, in any area, in the rate of cooling with respect to wearing modality (central: *DW* 0.16±0.08°C/sec vs. *CW* 0.17±0.07°C/sec; t= -0.22, p=0.83: Figure 6.6), or lens material (*all SiH* 0.16±0.08°C/sec vs. *etafilcon A* 0.18±0.08°C/sec; t= -0.91, p=0.37: Figure 6.7). Analysis of variance between balafilcon A, lotrafilcon A and etafilcon A revealed no significant differences in the rate of cooling between the groups in any of the areas across the ocular surface (central F=0.64, p=0.53).

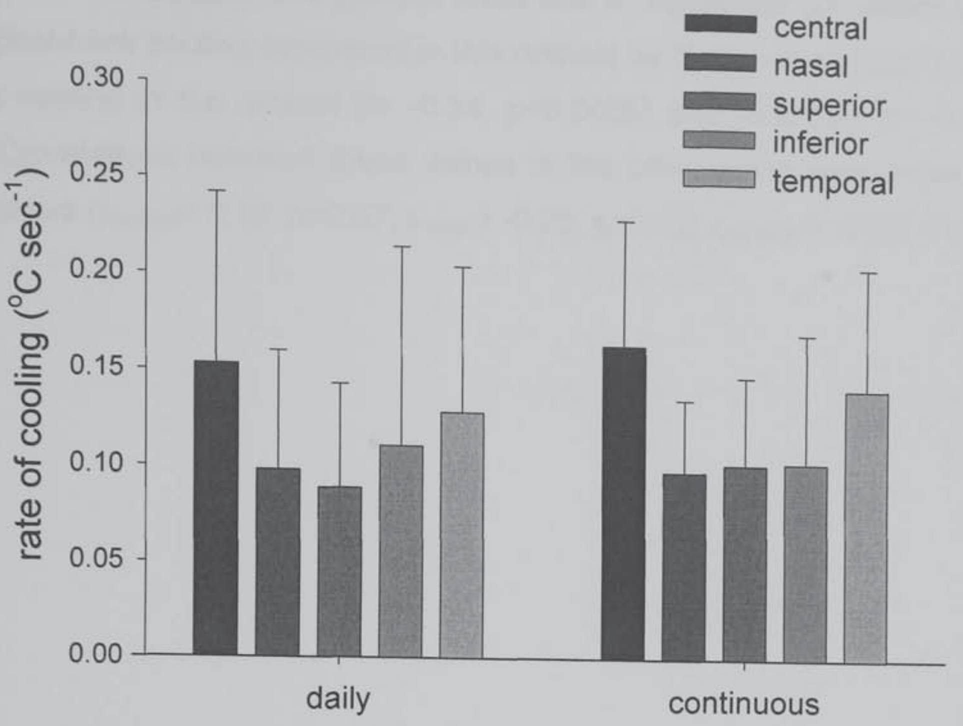


Figure 6.6
Rate of cooling post-blink comparing daily wear (n=24) with continuous wear (n=16)

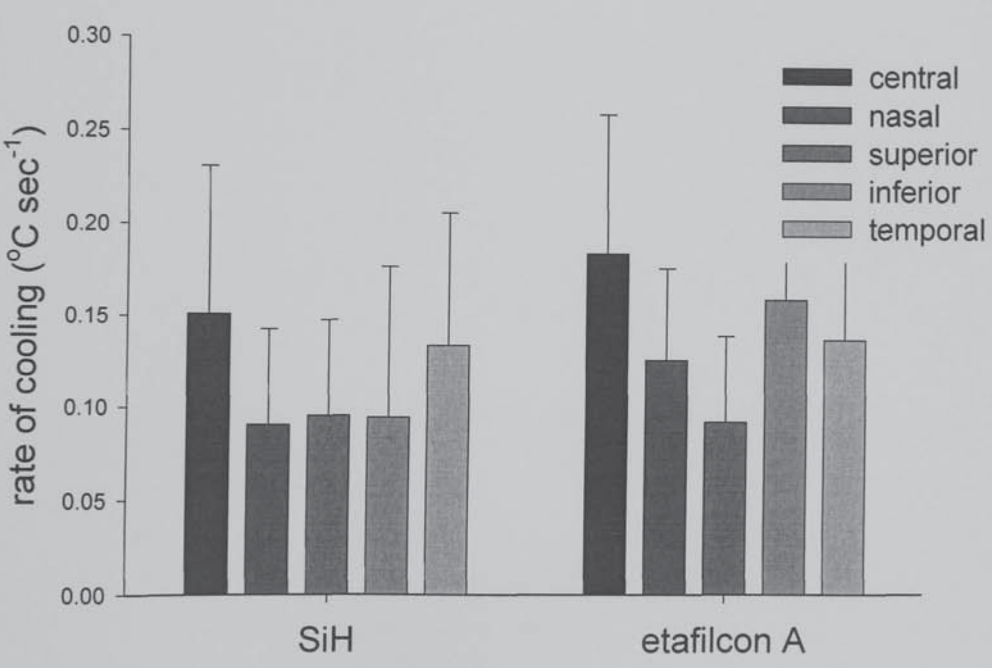


Figure 6.7
Rate of cooling post-blink comparing etafilcon A (n=8) with silicone hydrogel wearers (n=32).

Within the contact lens groups, there was a significant correlation between the rate of post-blink cooling (assessed in this respect by ¼ life values) and the magnitude of such cooling in the inferior ($r = -0.54$, $p<0.0005$) and temporal ($r = -0.42$, $p<0.01$) areas. Correlations between these values in the other areas were weak for contact lens wearers ($r_{central} = 0.10$, $p=0.57$; $r_{nasal} = -0.25$, $p=0.12$; $r_{superior} = -0.30$, $p=0.06$).

6.3.2 OST following removal of contact lenses

When contact lenses were removed, measured OST was greater in 100% of contact lens wearing subjects.

6.3.2.1 Initial temperature immediately post-blink

The initial measure of OST beneath the contact lenses was highly correlated ($r=0.97$; $p<0.0001$), but greater than (+0.62 ±0.3°C) that on top of the contact lenses (Figure 6.8). Similar correlations were observed across the other areas of the ocular surface: nasal $r=0.98$; superior $r=0.98$; inferior $r=0.95$; temporal $r=0.97$ ($p<0.0001$).

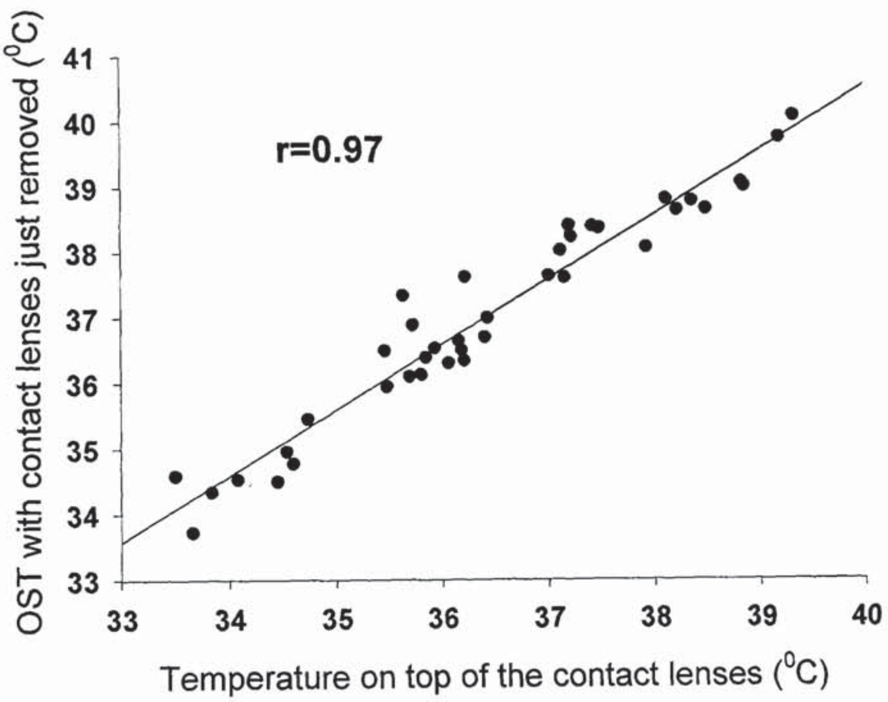


Figure 6.8
Relationship between initial OST beneath the lens and initial temperature on top of the contact lens (n=40; central area)

Initial OST under contact lenses was significantly greater compared to controls (37.1±1.7°C vs. 35.0±1.1°C; central, $p<0.005$) in all areas across the anterior eye surface (Figure 6.9).

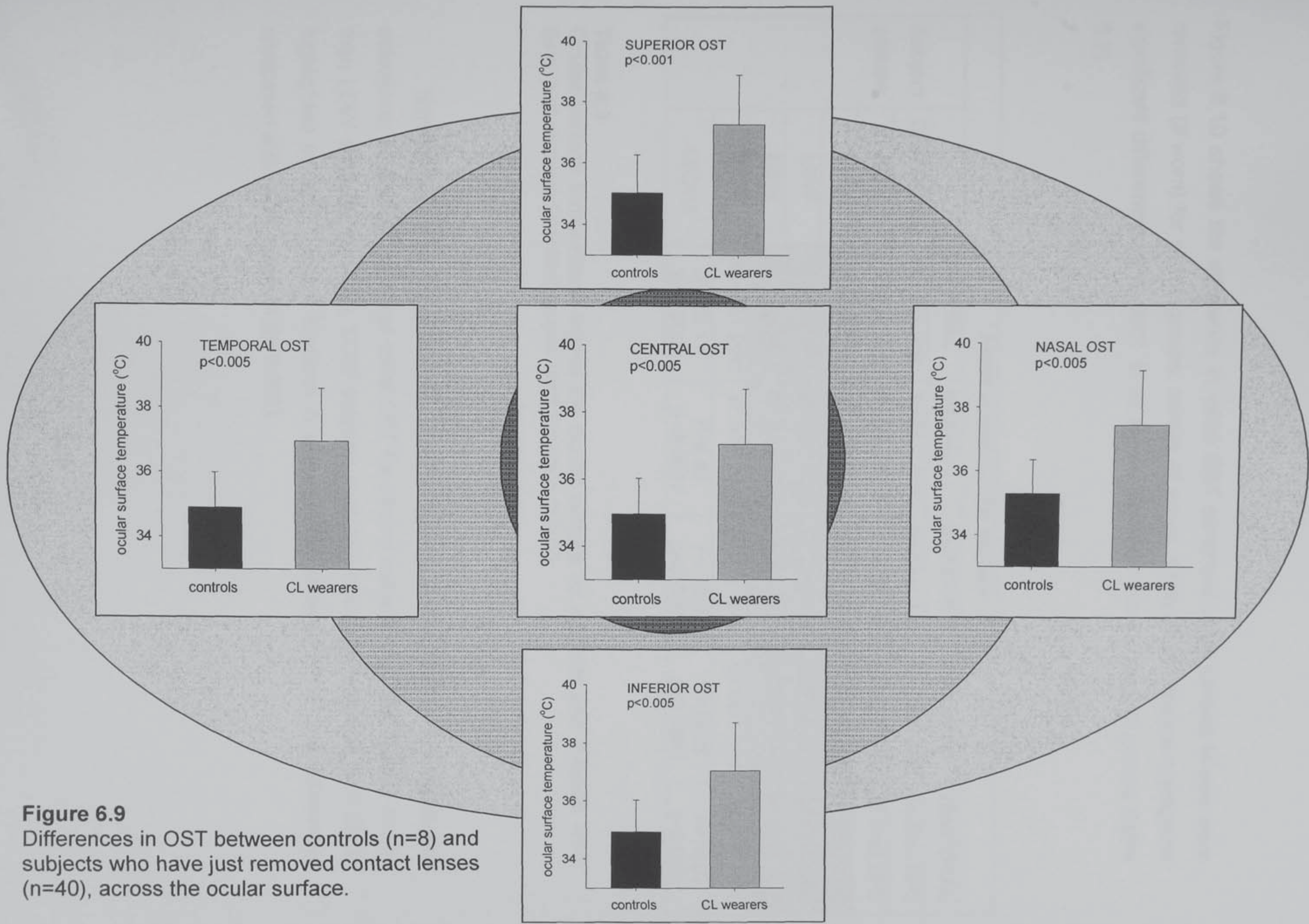


Figure 6.9
Differences in OST between controls (n=8) and subjects who have just removed contact lenses (n=40), across the ocular surface.

Figure 6.10 shows the difference in initial OST observed when contact lenses were removed (if worn) for all the groups, across all areas. Analysis of variance highlighted significant differences ($p<0.0001$ in all areas) between the six subject groups (table 6.9).

Areas across ocular surface						
		CENTRAL	**NASAL**	**SUPERIOR**	**INFERIOR**	**TEMPORAL**
Subject groups	**CONTROLS**	34.95±1.07°C	35.29±1.07°C	35.02±1.23°C	34.93±1.09°C	34.89±1.09°C
	EDW	35.28±1.09°C	35.52±1.18°C	35.48±1.10°C	35.20±1.04°C	35.24±1.05°C
	LDW	37.19±1.31°C	37.72±1.42°C	37.39±1.23°C	37.29±1.19°C	37.02±1.35°C
	LCW	38.61±1.03°C	38.94±1.00°C	38.72±0.91°C	38.58±0.89°C	38.57±0.83°C
	BDW	37.61±1.31°C	37.97±1.35°C	37.86±1.17°C	37.59±1.45°C	37.47±1.30°C
	BCW	36.60±1.55°C	37.04±1.59°C	36.87±1.74°C	36.58±1.65°C	36.52±1.63°C
	ANOVA	*F=10.13 p<0.0001*	*F=9.93 p<0.0001*	*F= 10.01 p<0.0001*	*F=10.35 p<0.0001*	*F=10.02 p<0.0001*

Table 6.9
Ocular surface temperature across the ocular surface for controls and subjects who have just removed contact lenses (n=8 for each group)

Scheffe post-hoc analysis was used to identify the source of the significant difference: in all areas average initial OST for control subjects was significantly cooler than LDW subjects ($p<0.05$), BDW subjects ($p<0.05$), and LCW subjects ($p<0.0001$; highlighted in table above). Etafilcon A wearers also showed significant differences compared with LCW subjects ($p<0.001$).

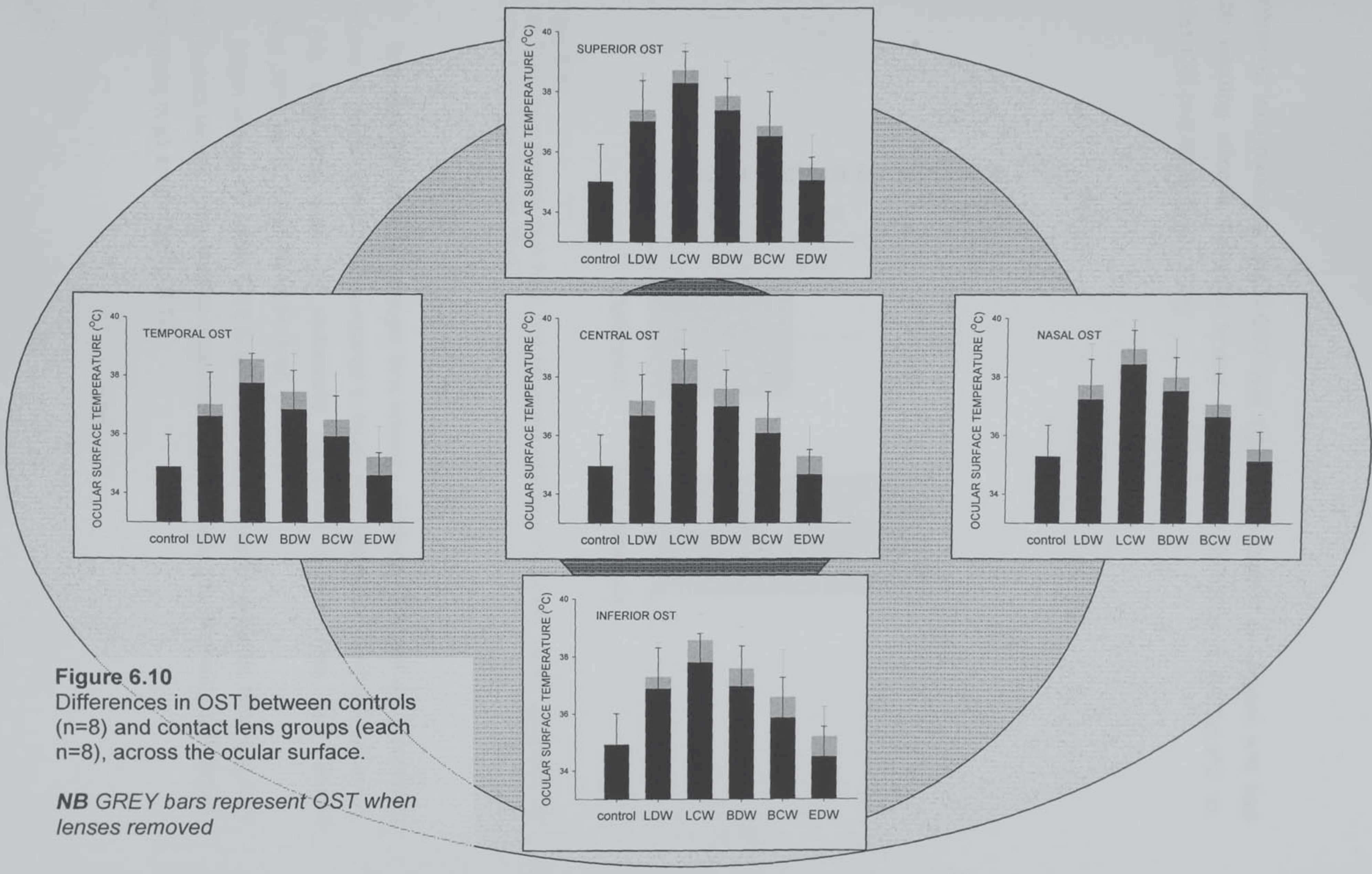


Figure 6.10
Differences in OST between controls (n=8) and contact lens groups (each n=8), across the ocular surface.

NB *GREY bars represent OST when lenses removed*

Comparing lens materials, initial OST was significantly greater in subjects who had been wearing silicone hydrogel lenses compared to Etafilcon A (37.5±1.5°C vs. 35.3±1.1°C; $p<0.0005$: figure 6.11).

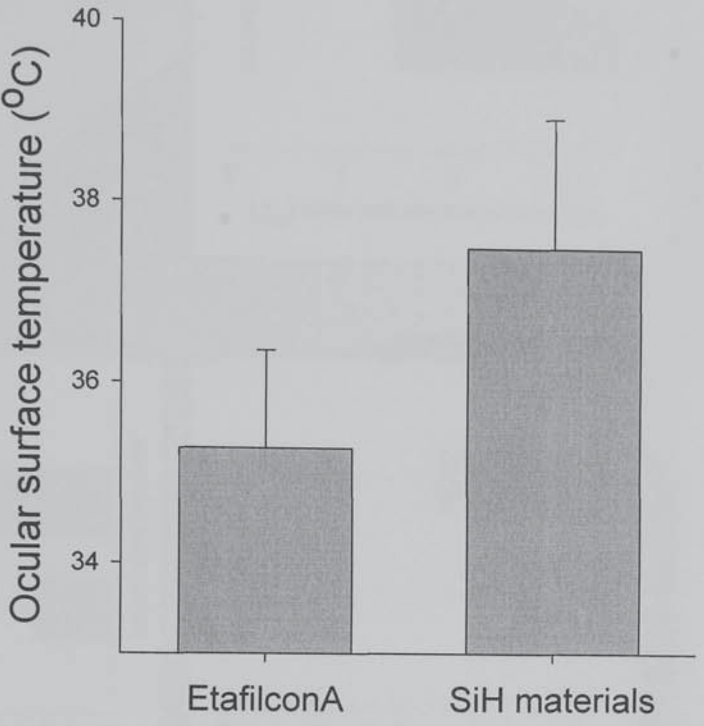


Figure 6.11
Initial OST in subjects that had just removed etafilcon A lenses (n=8) compared to silicone hydrogel lenses (n=32)

Between the silicone hydrogel lens wearers, subjects who had worn lotrafilcon A tended to have higher OST compared to those who had been wearing balafilcon A, but this difference was not statistically significant in any area (e.g. central 37.90±1.35°C vs. 37.07±1.49°C; t= -1.63, p=0.11).

Mean values for initial OST in all areas were lower for subjects removing daily wear contact lenses compared to continuous wear lenses: this difference approached significance in all areas (figure 6.12).

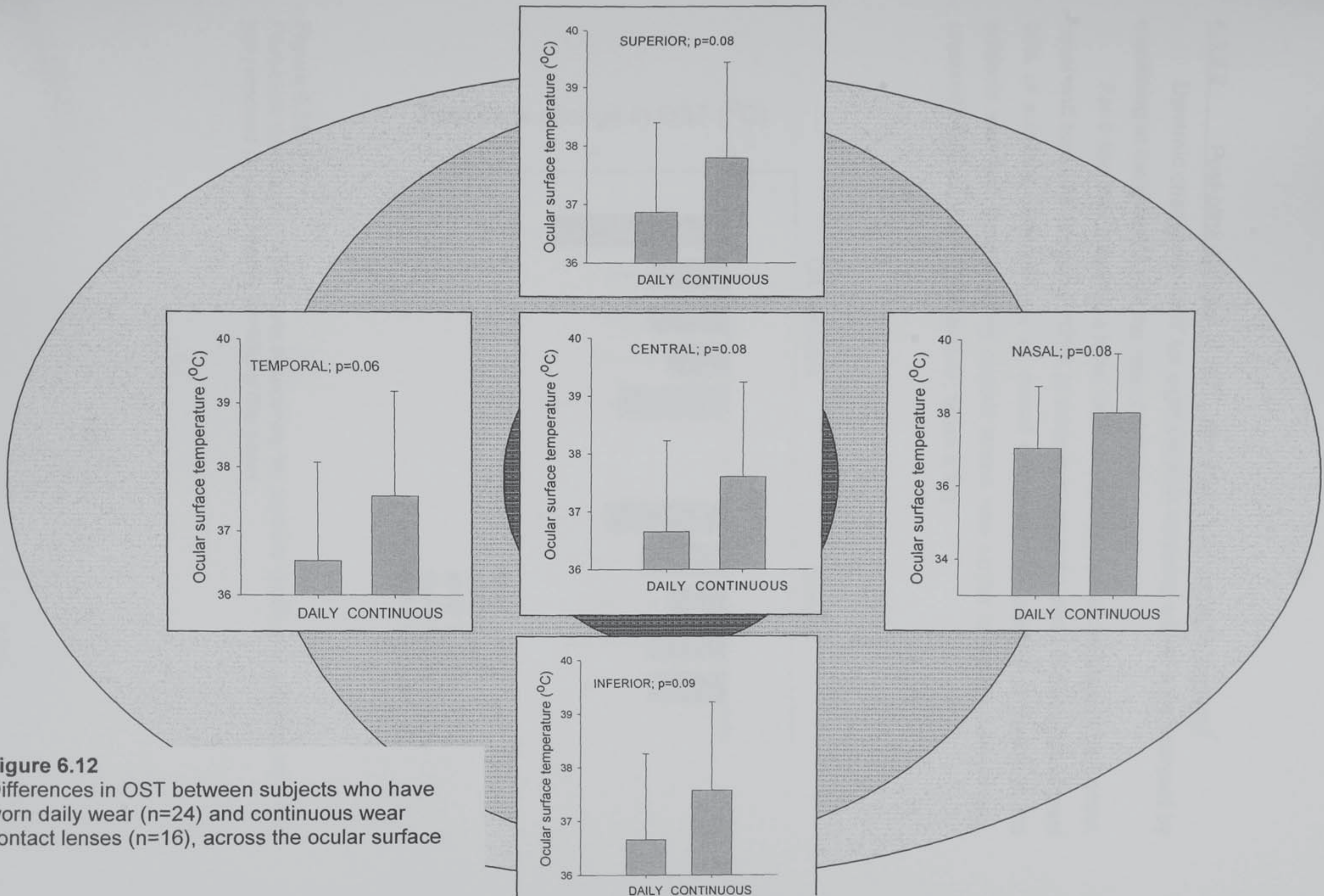


Figure 6.12
Differences in OST between subjects who have worn daily wear (n=24) and continuous wear contact lenses (n=16), across the ocular surface

6.3.2.2 Post-blink changes in OST, after contact lenses were removed

Dynamic changes in OST for eight seconds following a blink were analysed by examining at the *quantity* and the *rate of change*.

For 8 seconds following a blink, OST showed an overall decrease in the central, nasal and temporal areas for 100% of subjects: in superior and inferior areas at least 90% of subjects demonstrated an overall decrease. The quantity of cooling in the subjects who had been wearing contact lenses was similar to that of controls (e.g. unpaired t-test: central t=0.73, p=0.47; figure 6.13).

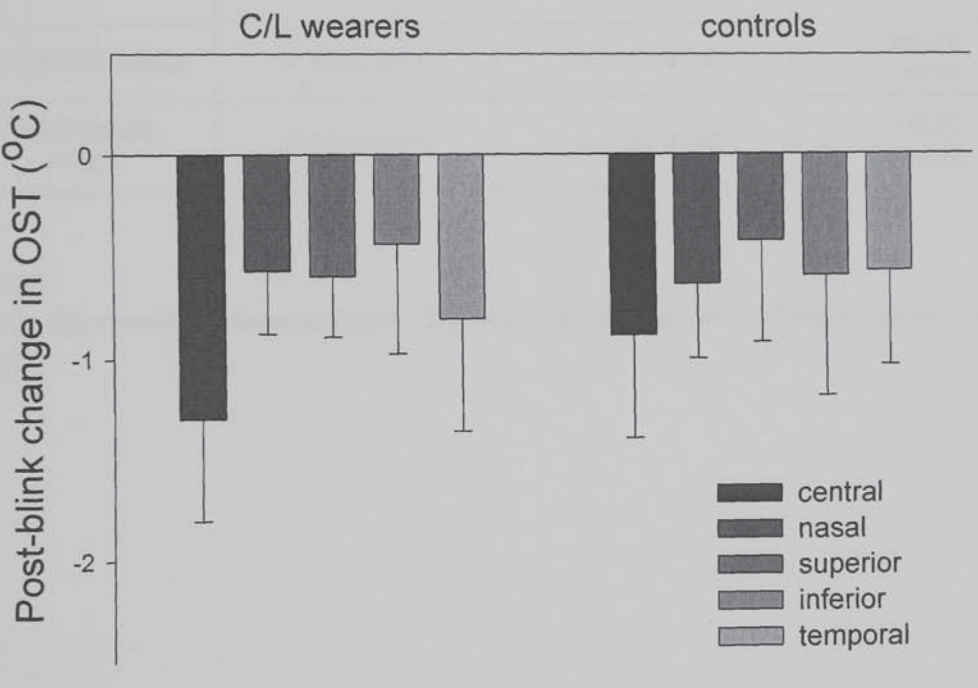


Figure 6.13
Post-blink decrease in OST over 8 seconds for controls (n=8) and subjects who had just removed contact lenses (n=40), in five areas

The quantity of post-blink cooling observed was greater in subjects removing daily wear lenses compared to continuous wear, in all areas across the ocular surface, but these differences were not significant statistically (table 6.10).

	DAILY WEAR(n=24)	CONTINUOUS WEAR (n=16)	*STUDENT'S T-TEST*
CENTRAL AREA	-1.12±0.55°c	-0.92±0.42°C	***t=1.24 p=0.22***
NASAL AREA	-0.59±0.35°c	-0.56±0.25°C	***t=0.30 p=0.77***
SUPERIOR AREA	-0.62±0.33°C	-0.57±0.24°C	***t=0.48 p=0.63***
INFERIOR AREA	-0.50±0.58°C	-0.36±0.47°C	***t=0.76 p=0.45***
TEMPORAL AREA	-0.88±0.61°C	-0.71±0.47°C	***t=0.93 p=0.36***

Table 6.10
Post-blink decrease in temperature, by area (grouped according to previous wearing modality)

Subjects who had worn etafilcon A lenses demonstrated greater amounts of post-blink cooling than the silicone hydrogel lens materials, in all areas, but this difference was only significant centrally (table 6.11).

	etafilcon A (n=8)	lotrafilcon A (n=16)	balafilcon A (n=16)	*ANOVA*
CENTRAL AREA	-1.46±0.52°C	-0.93±0.48°C	-0.93±0.44°C	*F=3.99;* ***p<0.05***
NASAL AREA	-0.68±0.39°C	-0.53±0.31°C	-0.56±0.26°C	*F=0.59;* ***p=0.56***
SUPERIOR AREA	-0.73±0.38°C	-0.54±0.30°C	-0.60±0.24°C	*F=1.03;* ***p=0.37***
INFERIOR AREA	-0.64±0.54°C	-0.42±0.59°C	-0.35±0.48°C	*F=0.78;* ***p=0.47***
TEMPORAL AREA	-1.10±0.66°C	-0.74±0.55°C	-0.73±0.48°C	*F=1.40;* ***p=0.26***

Table 6.11
Post-blink decrease in temperature, by area (grouped according to lens material previously worn)

The post-blink change in OST over 8 seconds was plotted for controls and subjects who had just removed contact lenses (Figure 6.14): the two groups had similar rates of post-blink cooling in all areas.

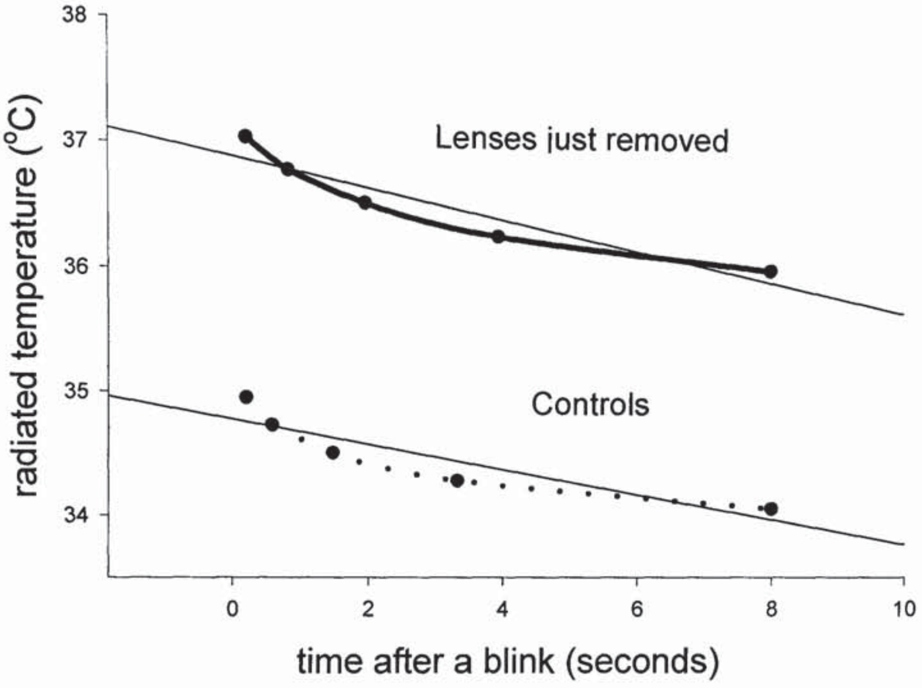


Figure 6.14
Post-blink change in OST for controls (*broken line*) and subjects who had just removed contact lenses (*solid line*)

Within the contact lens groups, there was no significant difference in the rate of cooling (as represented by ¼ life) with respect to wearing modality in any area (e.g. unpaired t-test for central area: $t=-0.93$, $p=0.36$).

Analysis of variance demonstrated no significant differences in the rate of cooling (as represented by ¼ life) according to contact lens material, in any area (e.g. centrally $F=0.46$, $p=0.63$).

6.4 Discussion

Previous studies have observed insignificant differences in measured OST during contact lens wear compared to non-wearers (Hill and Leighton, 1965a; Hamano et al., 1969; Fatt and Chaston, 1980). The superior sensitivity of the current instrumentation and greater subject numbers may explain the significant differences noted by this study. This study also used adapted wearers who had been wearing lenses for at least two hours: post-lens tear film (PoTLF) thickness has been shown to only stabilise after one hour of wear (Polse et al., 2002).

The results concur with a previous model (Martin and Fatt, 1986): there is a flow of heat from the eye to the environment, and due to the insulating properties of the contact lens material, there was an expected increase in temperature beneath a contact lens compared to a naked eye. Hydrogel and silicone hydrogel contact lenses will be less efficient than the cornea and the tear film, in terms of the absorption and emission of thermal radiation, due to their relative percentage water content (after Lerman, 1980). This accumulation of temperature beneath the lens will be transferred over time to the lens surface due to tear exchange and conduction/convection through the material (seen in Chapter 5). In this study, where lenses had been worn for at least two hours, radiation at the lens surface will occur (due to the temperature gradient), causing a temperature differential between post- and pre-lens tear film. This would explain the strong correlation between the recorded OST and temperature on top of the contact lenses. The characteristic thermal profile for initial OST straight after a blink (where the average temperature in the central area is coolest and nasal area was warmest), was observed both with and without hydrogel contact lenses: this finding concurs with the results from Chapter 4.

The main variables affecting this model of thermodynamics are likely to be the water content of the contact lens and the post-lens tear circulation. Post-lens tear mixing has been shown to be influenced by contact lens base curve radius (Lin et al.,

2003) and lens diameter (McNamara et al., 1999). This study observed significantly greater OST beneath silicone hydrogel contact lenses compared to etafilcon A lenses. Silicone hydrogel lenses have lower water content and significantly less volume of PoLTF, but more tear mixing, due to their high modulus of elasticity (Polse et al., 2002; Lin et al., 2003). The stiffness of these materials is a contributing factor to tarsal conjunctival hyperaemia and localised papillary conjunctivitis (Dumbleton, 2002): this hyperaemia may also contribute a warming effect on the ocular surface with each blink.

The closed eye environment is known to cause an increase in OST (Hill and Leighton, 1965a; Mapstone, 1968a; Fatt and Chaston, 1980; Martin and Fatt, 1986), and the PoLTF is rapidly reduced by eye closure (Nichols and King-Smith, 2003), most likely due to a combination of lid pressure and reduction in tear secretion (Sack et al., 1996). The results suggesting greater OST with continuous wear compared to daily wear might, therefore, be expected.

The observed decrease in OST following a blink has been previously suggested as a measure of tear film stability (Craig et al., 2000). Hydrogel lens wear is known to decrease tear film stability, especially when lenses have just been removed (Faber et al., 1991), and to increase tear-film evaporation (Cedarstaff and Tomlinson, 1983). Previous studies have not demonstrated any significant differences between materials for tear film evaporation rates and tear-thinning times (Cedarstaff and Tomlinson, 1983; Thai et al., 2002), but higher water content hydrogels have been shown to have longer non-invasive tear break-up times (Young and Efron, 1991). This study demonstrated greater and faster changes in temperature after a blink with contact lenses in situ, but did not appear to be affected by material or wear modality. This is in agreement with the latest research that shows that the pre-lens tear film thins significantly faster than the pre-corneal tear film (Nichols et al., 2004).

Clinical Relevance

The increased OST behind a hydrogel contact lens due to thermal insulation and limited tear mixing may contribute to known physiological responses in contact lens wear:

- Inadequate tear mixing is thought to play a part in adverse responses during extended wear of hydrogel contact lenses (Miller et al., 2003).
- Contact lens wear and/or eyelid closure show a suppression of normal physiological epithelial cell death (Ladage et al., 2002).
- Extended contact lens wear has been shown to cause loss of keratocytes (Efron et al., 2002)
- The post-lens environment has been likened to the closed eye environment: a sub-clinical state of inflammation where bacteria can accumulate and proliferate and there is routine activation of inflammatory responses (Sack et al., 2003). Vitronectin concentrations on hydrogel lens surfaces are greater for extended wear than for daily wear contact lenses, and are greatest at the posterior lens surface (Tighe et al., 1993; Tighe et al., 1998).
- Bacterial binding and protein deposition differs between organisms, lens materials and modalities of wear (Willcox et al., 2001). *In vitro* bacterial cultures of bacteria have been shown to adhere in the greatest numbers if grown under low temperature (25°C compared to 37°C) (Willcox et al., 2001): the greater OST with silicone hydrogel lens wear may have a beneficial effect in reducing the binding of such bacteria *in vivo*. Oxygen transmissibility has been shown by some studies to be inversely related to binding of Pseudomonas aeruginosa (Ren et al., 1999), although others have found no difference (Borazjani et al., 2004).

Silicone hydrogel lenses are being increasingly used as therapeutic contact lenses, for corneal protection and/or therapy (Kanpolat and Ucakhan, 2003; Montero et al., 2003), including post-refractive surgery where it has been shown that the eye already reaches relatively high temperatures (Betney et al., 1997; Maldonado-Codina et al., 2001). This is perhaps, slightly at odds with the idea of cooling as a universal principle in medicine (Fujishima et al., 1995; Schrage et al., 1997), but it is likely that the superior oxygen permeability of these materials helps to maintain corneal physiology and promote healing despite high temperature.

6.5 Conclusions

OST increases when contact lenses are worn for at least two hours, the effect being greater with silicone hydrogel lenses compared to etafilcon A, and tends to be greatest if lenses have been worn continuously. The magnitude and rate of post-blink cooling increases when lenses are worn, but they do not appear to be affected by lens type or wear modality.

CHAPTER 7

THE DYNAMIC EFFECT OF ARTIFICIAL TEAR SUBSTITUTES ON OCULAR SURFACE TEMPERATURE

7.1 Introduction

The maintenance of a sufficient and stable tear film on the pre-ocular surface is a requirement for the well-being and efficient performance of the eye (Trees and Tomlinson, 1990). Disorders of the tear film (often called 'dry eye syndrome') are amongst the most common ocular abnormalities (Schein et al., 1997), and the most complex (Johnson and Murphy, 2004). The prevalence of dry eye disorders has been estimated at 20% in adults over 45 years of age (Brewitt and Sistani, 2001): they result from either decreased tear production or excessive tear loss from evaporation. The most widely used therapeutic approach for dry eye is tear replacement and retention by topical artificial tear drops (ATDs), and although palliative rather than curative, is frequently effective in providing relief. However, artificial tear formulations are not all alike, and treatment is often based on a trial and error method for each patient. Such preparations fall into three main groups:

1) Aqueous artificial tears - these are flowing liquids with low viscosity that are designed to replace or replenish the tear aqueous component. The active ingredients to increase viscosity include cellulose derivatives (such as methylcellulose, hydroxyethylcellulose, and carboxymethylcellulose; their effect tend to be short lasting, however), polyvinyl alcohol (PVA; acts mainly as a wetting agent), and polyvinylpyrrolidone (PVP).

2) Ocular lubricants - these are ointments that reduce friction between lid and globe. Active ingredients include white soft paraffin, liquid paraffin and lanolin alcohol.

3) Viscoelastics - these are the formulations that exhibit both liquid and gel properties: they become a fluid when agitated and set again when left to rest. Active polymers include polyacrylic acid (carbomers) and naturally-occurring sodium hyaluronate.

In addition to the high molecular weight polymers used to increase viscosity, artificial tear formulations include buffers to maintain pH (slightly alkaline is best for dry eye (Norn, 1988), sodium chloride to maintain tonicity, and preservatives to kill or inhibit micro-organisms.

Much of the literature surrounding ATDs has focused on clinical trials based on subjective comfort ratings, or an objective determination of drop retention or residence times. A selection of objective techniques from the literature is summarised in Table 7.1.

Various problems exist with these methods. Any method that adds bulk to the ATD formulation has the potential to displace more of the natural tear fluid and vital surface oils, which will in turn, affect the performance of the ATD (Burstein, 1985).

Invasive techniques, such as those using fluorescein, are hampered by their tendency to induce reflex tearing, leading to short retention times. Measurement of tear break-up times (both fluorescein and non-invasive) generally show large variation within subjects (Korb, 2000), although Lemp and colleagues found their subjects to be remarkably constant in this respect in their 1975 paper. Studies using tracers (scintigraphic methods or fluorescein) assume that the movement of the polymer parallels the tracer with which it is in solution; this may not be the case as they are not 'bound' together (Ludwig et al., 1992). Other indirect measures of the efficacy of ATDs include assessing ocular penetration of dyes (Gobbels and Spitznas, 1991), pachymetry pre- and post-ATD instillation (Shimmura et al., 1998), the use of contact thermistors to detect anti-inflammatory properties of ATDs on the eyelids (MacKeen and Roth, 2002), assessment of tear film evaporation rates (Trees and Tomlinson, 1990) and monitoring their excretion through the naso-lacrimal apparatus (Linn and Jones, 1968).

AUTHOR & DATE	***TECHNIQUE***	***ATD FORMULATIONS USED***	***SUBJECTS***	***FINDINGS***
Lemp et al (1975)	Serial break-up time (BUT) measures	13 brands including Liquifilm, Tears Naturale, Adapt; one eye only	12 normals	All solutions lengthened BUT, solutions using PVA or cellulose ethers lasted 35-60mins; solutions using hypromellose lasted 90mins
Holly (1978)	*In vitro* study looking at effect of ATDs on lipid layer of artificial tear film	Various including Liquifilm & Tears Naturale 1	*in vitro*	Liquifilm stabilized the lipid layer better than Tears Naturale I, due to use of preservative benzylkonium chloride (BAK) in latter.
Trees & Tomlinson (1990)	Tear evaporation rates	Liquifilm, Tears Naturale II and saline as control; one eye only	9 normals	Initial increase in evaporation rate suggesting *unstable* tear film, followed by return to baseline: may result from use of ATDs with mucin-like properties rather than lipid-like properties.
Gobbels & Spitznas (1991)	Fluorophotometry to assess corneal permeability of dry eyes with ATD therapy	Polyvinyl alcohol (PVA) - 1.4% Polyvinylpyrrolidone (PVP) -2%	68 dry eye subjects	Permeability decreased after treatment with PVP & PVA, except when BAK preservative was present.
Greaves et al (1991)	Quantitative gamma (γ) scintigraphy to measure clearance rates of ATD	PVA Hydroxypropylmethylcellulose (HPMC, Hypromellose)	12 normals; 12 dry eye subjects	No significant difference in clearance between solution studied
Snibson et al (1992)	Quantitative gamma (γ) scintigraphy to measure ½ lives	PVA - 1.4% Sodium Hyaluronate (SH) - 0.2% HPMC (Hypromellose) - 0.3% One eye only	5 dry eye subjects	SH remains in contact significantly longer than both PVA and HPMC
Ludwig et al (1992)	Fluorescent tracers and slit-lamp fluorophotometry	Purpose made solutions containing cellulose viscolysers to one eye	5 normals	Large variation between subjects for any solution, but higher viscosity tends to slow clearance of fluorescein. Noted that reaction to buffer used can accelerate drainage.
Shimmura et al (1998)	Orbscan differential pachymetry maps pre- and post-ATD instillation	SH - 0.3% Buffered NaCl; one eye only	8 normals	Uneven thickening of the tear film, particularly superiorly.
Pascuale et al (2004)	Analysing tear film interference (TI) images	Emulsion eye drop (Refresh Endura) ; saline in other eye as control	5 normals; 10 dry eye subjects	Increased lipid film thickness
MacKeen & Roth (2002)	Thermistors against closed lids to assess anti-inflammatory effect of ATDs	Various including PVP, PVA, saline and lid-delivered calcium carbonate ointment	544 eyes from 272 dry-eye subjects	Calcium carbonate ointment appeared to lower lid temperature for longer

Table 7.1
A selection from the literature concerning objective measurement techniques for retention times/ efficacy of artificial tear drops (ATDs)

It was this review of the literature and also the fact that sufferers of dry eye often use the descriptors 'burning' and 'dry' when describing their symptoms (Bandeen-Roche et al., 1997), that led to the rationale for this study. Thermography has been seldom used in dry eye studies previously: artificial tears of various temperatures were shown to lower ocular surface temperature (OST) and improve subjective comfort (Fujishima et al., 1997). In his thesis, Morgan looked at the effects of 1.4% polyvinyl alcohol compared to saline at room temperature. Both reduced OST for the first two minutes, and then OST returned to baseline after 10 minutes: PVA reduced OST to a greater degree than saline, but not significantly (Morgan, 1994).

The purpose of this study was to utilize the unique, non-invasive and objective capabilities of the thermal camera (described in Chapter 2.1) to examine *dynamically* the temperature effects of instilling different ATDs in a group of normal subjects.

7.2 Materials and methods

7.2.1 Subjects

Fourteen young volunteers were recruited for this study (5M, 9F; age 24.8±3.8yrs). For the sake of homogeneity between subjects only normal healthy individuals were included in this study. Inclusion criteria for the subjects were:

- no current use of medication, topical or systemic
- no symptoms of dry eye
- no external eye disease
- not wearing cosmetics at the time of the examination
- no contact lens wear for at least 48hours

Informed consent was obtained from each subject and all procedures conformed to the declared tenets of Helsinki. Subjects were randomized to receive each of the five formulations at a weekly visit to the laboratory, with each laboratory session at the same time of day. Sessions were separated by seven days in order to eliminate any risk of persistent effects of the ATD on the ocular surface.

7.2.2 Materials

Five formulations of artificial tear solutions (four commercially available and a saline control) were used (table 7.2).

BRAND NAME	MANUFACTURER	ACTIVE INGREDIENT	PRESERVATIVE	BUFFER
LIQUIFILM®	Allergan, High Wycombe, UK	polyvinyl alcohol (PVA) 1.4%	Benzalkonium chloride (BAK)	phosphate/ acetate Hypotonic
HYPROMELLOSE	Martindale pharmaceuticals Ltd, Romford, UK	Hypromellose 0.3%	Benzalkonium chloride (BAK)	boric acid
HYLOPROMPT®	Spectrum Ophthalmics , Macclesfield, UK	Sodium hyaluronate (SH) 0.1%	none - uses novels ophthalmic delivery system	phosphates; isotonic
VITAL EYES®	CIBA Vision, Southampton, UK	Methylhydroxypropylcellulose (MHPC) (Vit A, vit E)	Benzalkonium chloride (BAK) Disodium edetate (EDTA)	boric acid
Saline MINIMS®	Chauvin Pharmaceuticals Ltd, Romford, UK	Sodium chloride 0.9%	none; single dose eye drops	isotonic

Table 7.2
Artificial tear preparations used in this study

7.2.3 Instilling the artificial tears

Due to visible differences in the bottles, the experiment was only masked to the subjects. The solutions were instilled with an adjustable micropipette with disposable tips to ensure similar drop size (50±2µl - consistent with the typical amount of fluid dispensed). The tip of the pipette was discarded after each use and replaced with a clean one. The drop was placed into the central, lower fornix. At each session subjects received a controlled drop of one of the five preparations to first their left eye, then the right eye. Thermography was *only* performed on the right eye but preliminary work indicated that subjects felt more comfortable with drops instilled into both eyes. Subjects were instructed to keep their eyes closed for two seconds after each drop was instilled, as prolonged eye closure retards tear drainage (White et al., 1991).

7.2.4 Experimental procedure

With each subject, the measurements were carried out in a logical, stepwise manner: slit-lamp examination of each subject before each session allowed the subjects to suitably adapt to room conditions (temperature 21.4±0.9°C, 40±1% humidity). OST of the right eye was dynamically recorded for eight seconds following a blink with the Thermo Tracer TH7102MX (as described in Chapter 2.14), before instillation of the drop. The average of three readings was calculated. A randomised, controlled drop was then instilled into the left eye followed by the same to the right eye. Dynamic temperature recordings of the right eye (eight seconds post-blink) were then made at 10s, 20s, 30s, 40s, 50s, and then every minute for fifteen minutes.

7.2.5 Data collection and analysis

For each recording, temperatures were recorded from 9 points across the central area of ocular surface, as described in Chapter 2.13. All temperature changes were normalized to the baseline of pre-drop OST for each subject at each session. The data management template in Excel® (described in Chapter 2.14) was used to calculate the main effects: initial OST post-blink and the magnitude of the decrease in OST following a blink over eight seconds.

The relationship between pre-drop OST and initial post-drop OST was investigated using Pearson's correlation coefficient. A two-way repeated measures ANOVA was conducted to compare OST at selected time intervals following drop instillation (factors being drop and time).

7.3 Results

7.3.1 Initial effect on OST (10s after instillation)

There was a strong positive correlation between pre-drop OST and post-drop OST at 10s (r=0.96, n=14x5, p<0.001; Figure 7.1).

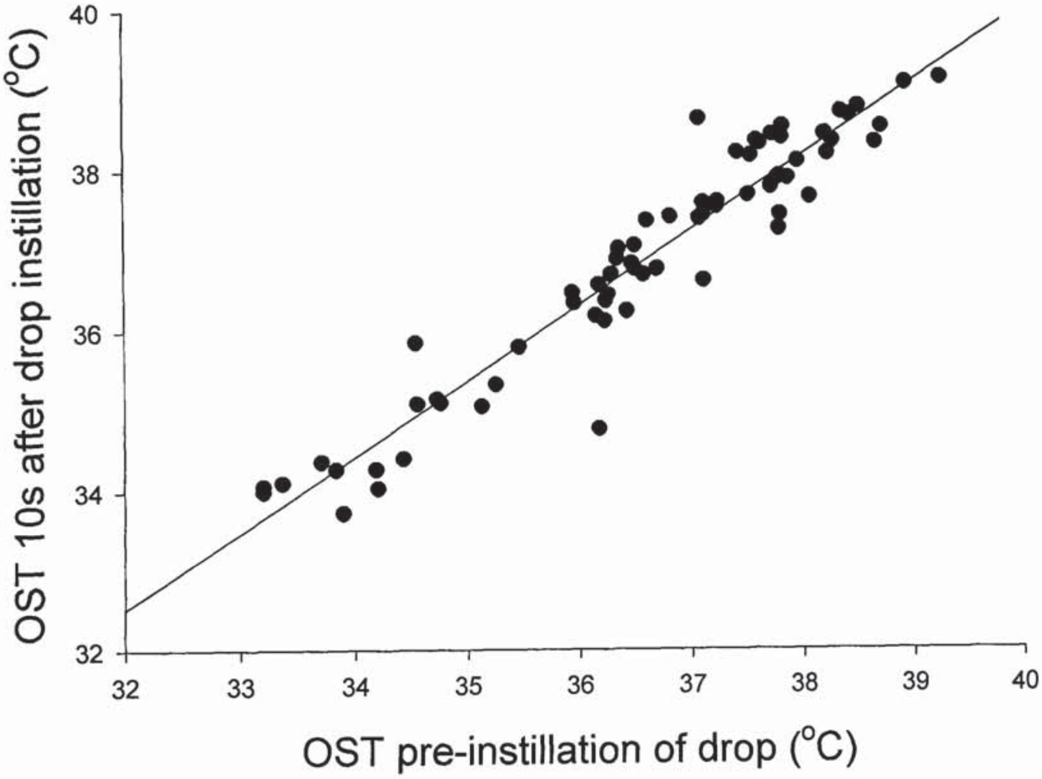


Figure 7.1
Correlation between pre-drop OST and OST 10s after drop instillation (n=14x5)

A significant *increase* in measured OST (straight after a blink) at 10 seconds after drop instillation was observed in over 81% of cases (average increase +0.33±0.4°C; paired t-test: t=-6.39, p<0.0005; Figure 7.2).

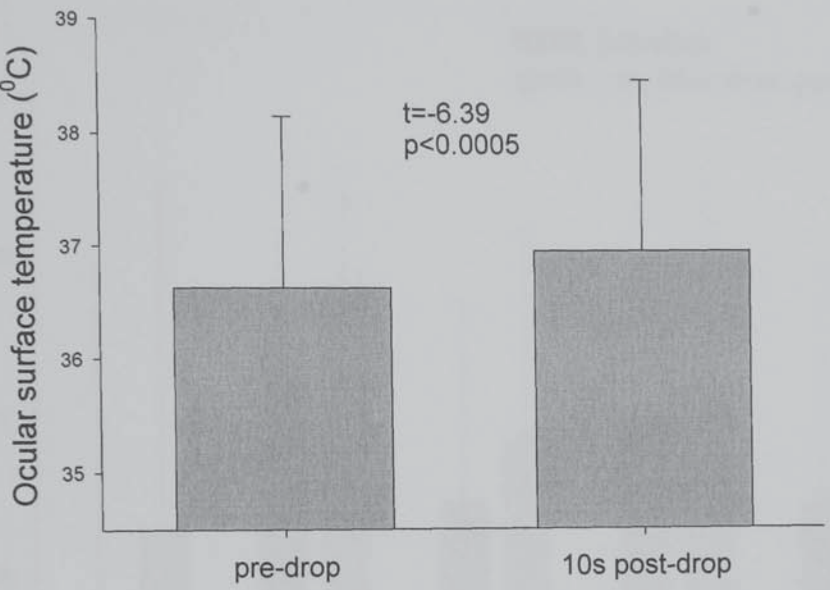


Figure 7.2
Mean OST pre-drop instillation and 10s after drop instillation (measured 0.2s after a blink; n=70). Error bars=1SD.

At 10s, there was no significant difference in the OST (straight after a blink) between the drops used (one-way repeated measures ANOVA F=1.42, p=2.41; Figure 7.3).

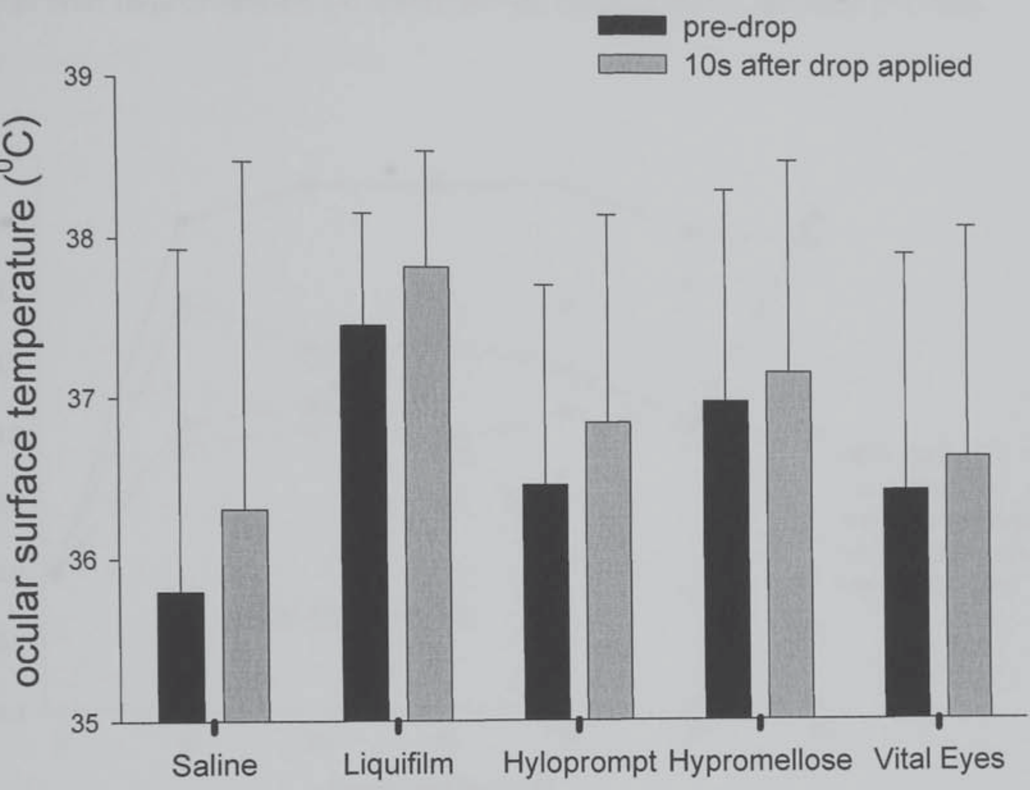


Figure 7.3
Change in OST after five different ATDs are instilled (n=14, crossover design, error bars=1sd)

7.3.2 Effect on OST for first minute after drop instillation

The average changes in OST (normalised to baseline) over the first minute for the five ATDs are shown in Figure 7.4. Significant differences between drop type emerge after one minute (one-way repeated measures ANOVA $F=3.72$, $p<0.01$): post-hoc analysis revealed the change in OST after one minute Vital Eyes to be greater than that with hypromellose (-0.09±0.34° vs. 0.52±0.46°C; Scheffe $p<0.05$).

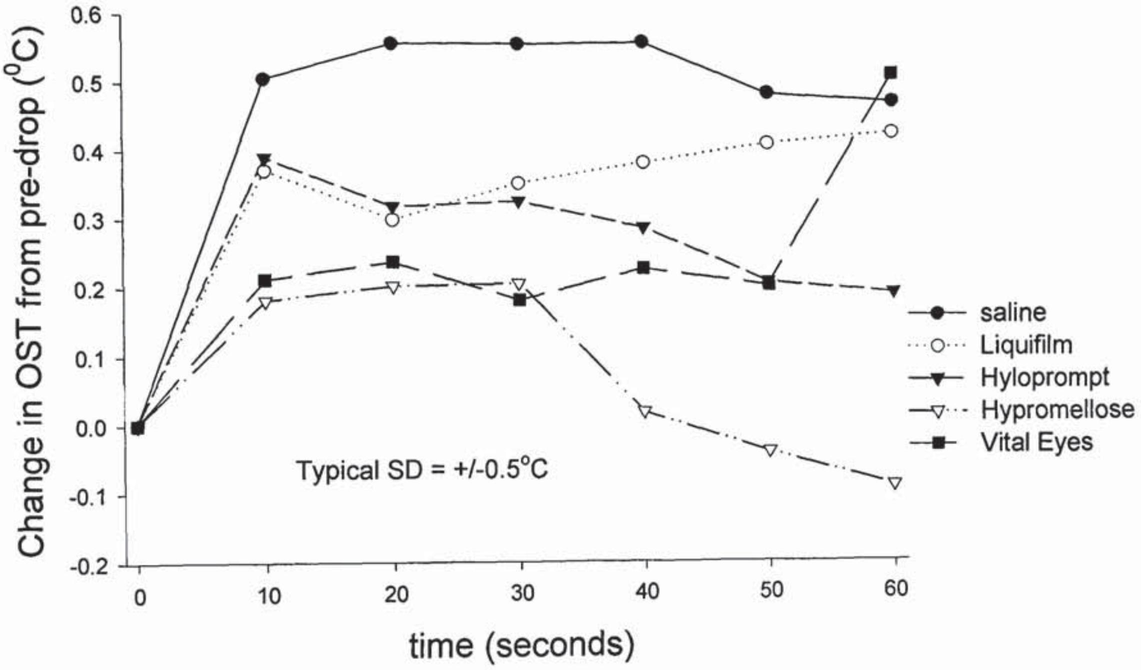


Figure 7.4
Change in OST over the first minute, following instillation of artificial tears
(Error bars removed for clarity: typically ±0.5°C)

7.3.3 Effect on OST over fifteen minutes after drop instillation

The average change in OST over fifteen minutes for each drop type is shown in Figure 7.5; error bars represent 1 SEM (standard error of the mean).

Two-way repeated measures ANOVA revealed that both time ($F=2.42$; $p<0.005$) and drop type ($F=27.58$; $p<0.0001$) were significant factors in OST overall (with no significant interaction between the two factors: $F=0.56$; $p=0.99$). Post-hoc comparisons (Scheffe) indicated the OST with hypromellose and Hyloprompt drops was significantly different to Liquifilm, saline and Vital Eyes ($p<0.005$ for each comparison). There was a tendency for the application of hypromellose to produce least change in OST with time, and Vital Eyes to produce the most. A one-way, repeated measure ANOVA, with post-hoc testing, was used to reveal the significant differences between the drops at specific time intervals. These results, along with means and standard deviations are shown in Table 7.3.

Significant differences in the change in OST with different drop types were observed at 1-5 minutes post-instillation: post-hoc analysis showed that the significance arose namely from differences between hypromellose and saline, and hypromellose and Vital Eyes® (e.g. at 1 min: hypromellose vs. Vital Eyes Scheffe, $p<0.05$). Other time points showed no statistically significant differences between drop types.

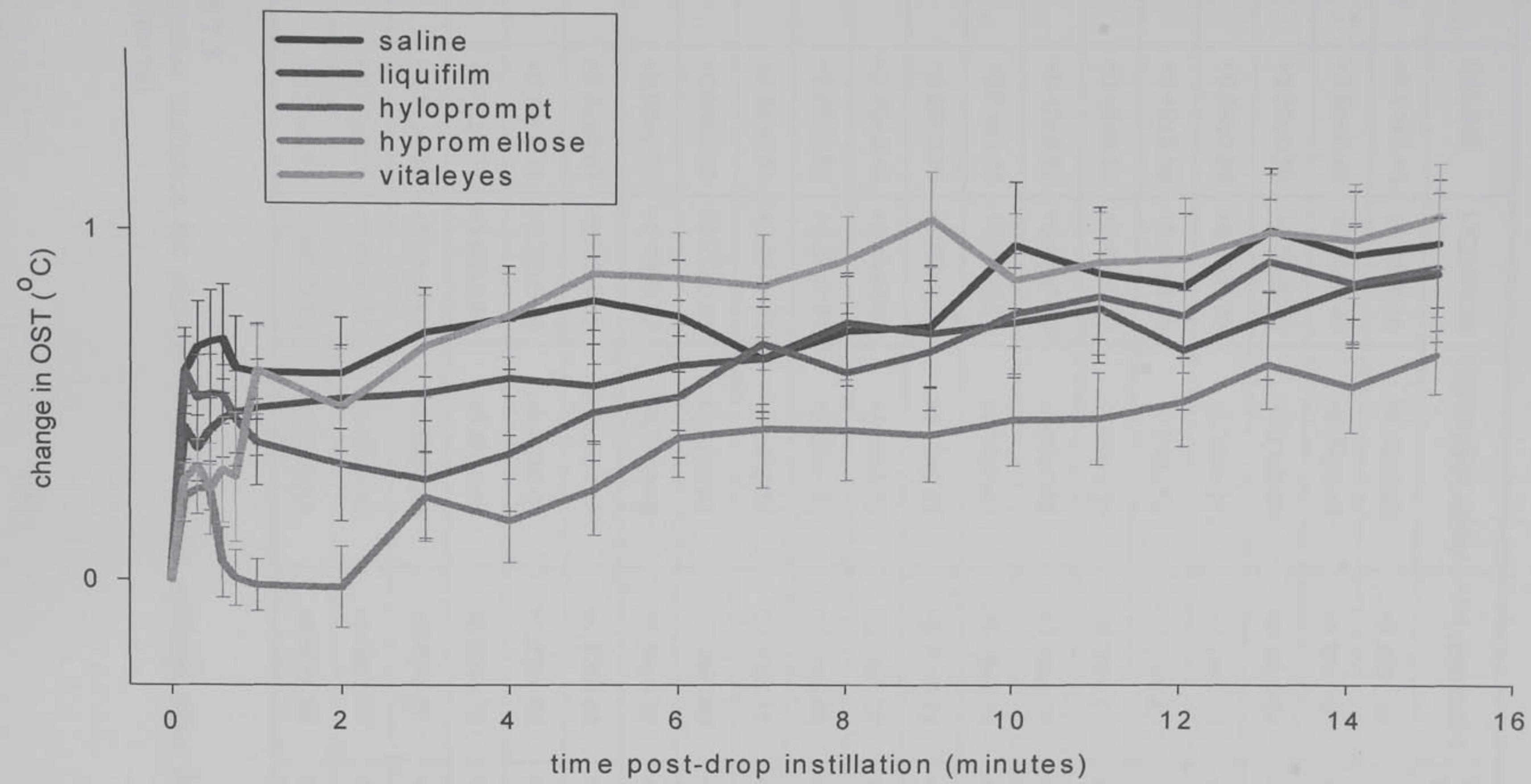


Figure 7.5
Change in OST over 15 minutes, following instillation of artificial tears (0 represents baseline temperature pre-drops) [Error bars represent 1 SEM]

TIME PERIOD	SALINE	LIQUIFILM	HYPROMELLOSE	HYLOPROMPT	VITAL EYES	*ANOVA RESULTS (n=14)*
10sec	+0.51±0.46	+0.37±0.40	+0.18±0.29	+0.39±0.64	+0.22±0.26	*F=1.419 p=0.24*
20	+0.56±0.49	+0.30±0.43	+0.20±0.30	+0.32±0.67	+0.24±0.33	*F=1.579 p=0.19*
30	+0.56±0.56	+0.36±0.42	+0.21±0.28	+0.33±0.64	+0.19±0.47	*F=1.657 p=0.17*
40	+0.56±0.59	+0.36±0.48	+0.02±0.37	+0.29±0.70	+0.23±0.53	*F=2.199 p=0.08*
50	+0.49±0.56	+0.41±0.48	-0.04±0.33	+0.21±0.81	+0.21±0.64	*F=2.082 p=0.10*
1min	+0.48±0.53	+0.43±0.53	-0.09±0.34	+0.19±0.71	+0.52±0.46	*F=3.718 p<0.01*
2	+0.49±0.57	+0.45±0.59	-0.09±0.48	+0.15±0.77	+0.36±0.65	*F=2.981 p<0.05*
3	+0.61±0.48	+0.47±0.64	+0.16±0.51	+0.09±0.78	+0.52±0.62	*F=3.028 p<0.05*
4	+0.65±0.53	+0.52±0.62	+0.10±0.48	+0.17±0.74	+0.63±0.51	*F=4.347 p<0.005*
5	+0.69±0.49	+0.49±0.62	+0.19±0.51	+0.24±0.80	+0.65±0.62	*F=3.003 p<0.05*
6	+0.65±0.62	+0.57±0.67	+0.32±0.50	+0.33±0.82	+0.65±0.70	*F=1.316 p=0.27*
7	+0.56±0.51	+0.59±0.62	+0.38±0.61	+0.46±0.81	+0.68±0.64	*F=0.646 p=0.63*
8	+0.59±0.59	+0.70±0.51	+0.38±0.53	+0.38±0.80	+0.79±0.56	*F=1.942 p p=0.11*
9	+0.60±0.65	+0.70±0.52	+0.37±0.48	+0.45±0.79	+0.90±0.56	*F=2.327 p=0.07*
10	+0.83±0.67	+0.70±0.56	+0.43±0.47	+0.53±0.83	+0.75±0.67	*F=1.439 p=0.23*
11	+0.74±0.71	+0.73±0.58	+0.42±0.47	+0.58±0.80	+0.78±0.60	*F=1.136 p=0.35*
12	+0.68±0.77	+0.63±0.61	+0.45±0.49	+0.52±0.75	+0.82±0.60	*F=1.193 p=0.33*
13	+0.81±0.74	+0.71±0.64	+0.55±0.48	+0.65±0.87	+0.87±0.61	*F=0.750 p=0.56*
14	+0.77±0.70	+0.83±0.60	+0.50±0.48	+0.58±0.90	+0.88±0.56	*F=1.359 p=0.26*
15	+0.78±0.73	+0.83±0.58	+0.58±0.43	+0.65±0.93	+0.90±0.58	*F=0.895 p=0.47*

Table 7.3
Descriptive statistics for change in OST for 15 minutes after instillation of artificial tears (n=14)

7.3.4 Effect on magnitude of post-blink cooling after drop instillation

Chapter 4 demonstrated a mean decrease in OST following a blink of 0.68±0.5°C, for 200 normal subjects. After the application of artificial tears, an overall decrease in OST was still observed in all but 22 out of 1330 experimental runs, i.e. in over 98% of cases a post-blink decrease in OST occurred.

The magnitude of the post-blink cooling (over 8s) was significantly greater after drops were inserted (*pre-drops* -0.50±0.21°C vs. *at 10s* -0.65±0.15°C; paired t-test t= -3.28, p<0.005).

Vital Eyes appeared to have the greatest effect, i.e. more post-blink cooling, but differences between drop types were statistically insignificant, due to large variance (one-way repeated measures ANOVA F=1.22, p=0.32; Figure 7.6).

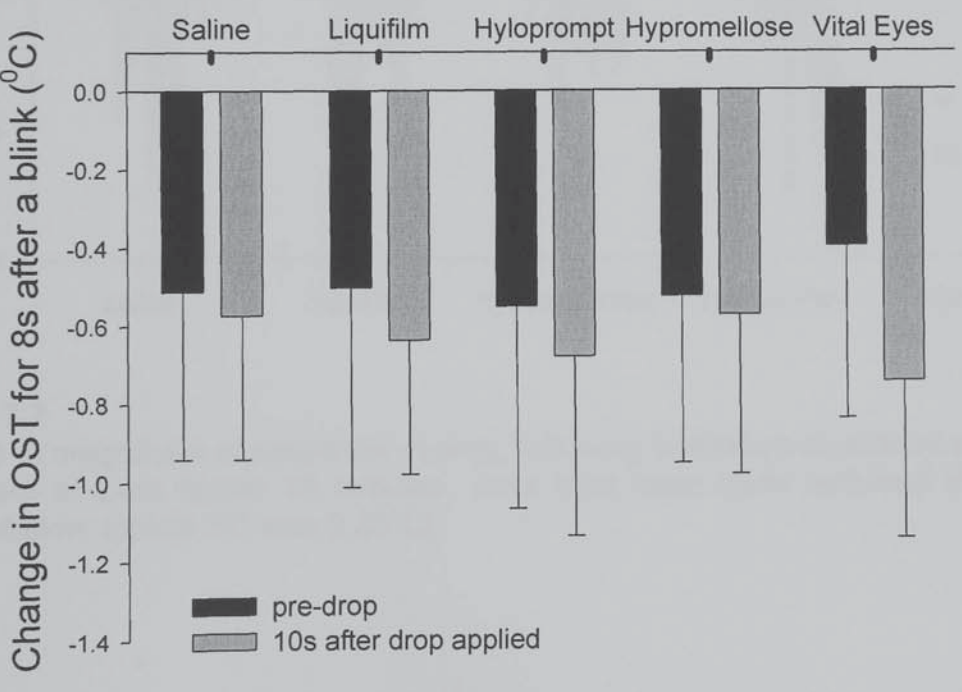


Figure 7.6
Magnitude of post-blink cooling before and 10s after drop instillation, for different ATDs (n=14 crossover design, error bars=1sd)

The changes in the magnitude of the post-blink cooling over time, with different drop type, are shown in Figure 7.7.

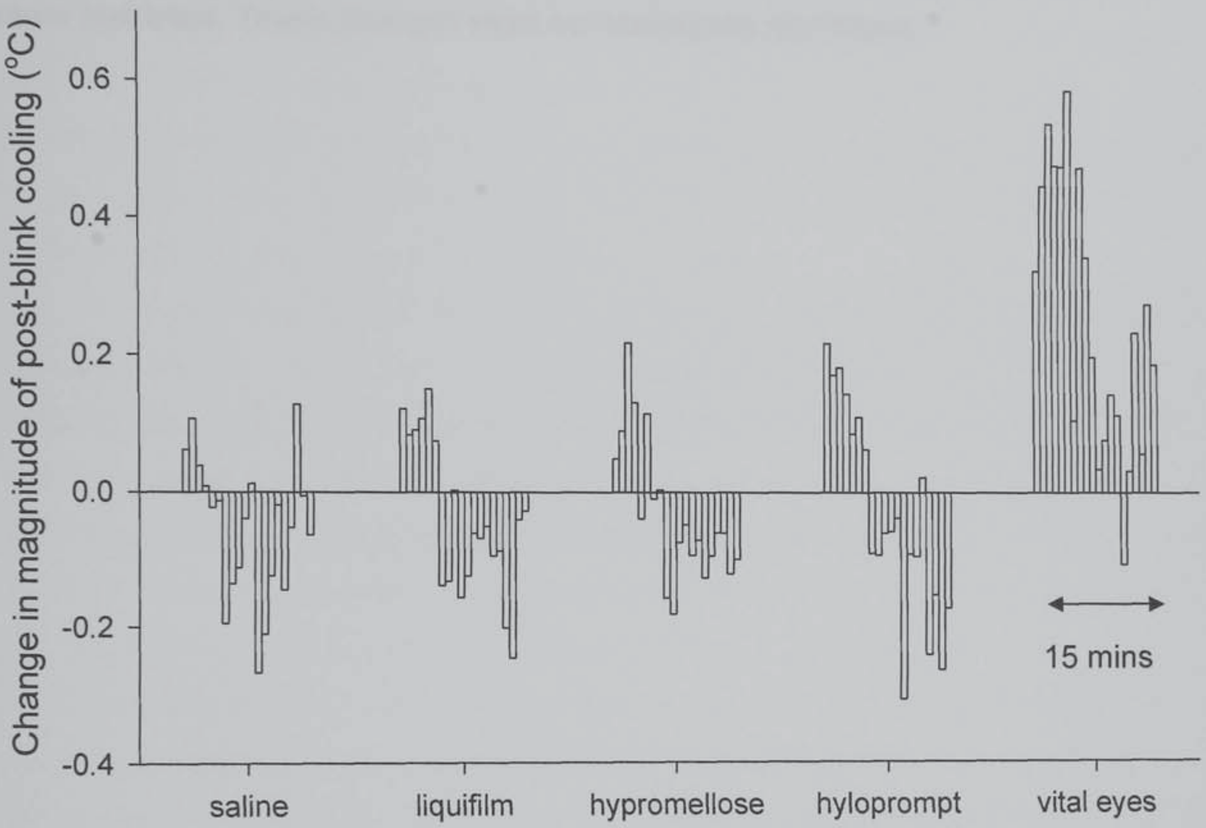


Figure 7.7
Change in magnitude of post-blink cooling, following instillation of artificial tears [Each set of bars spans 15 minutes; error bars have been removed to aid visual interpretation; typical SD was 0.35°C]

For the first minute, the effect on the magnitude of post-blink cooling differed significantly between drop types (repeated measures ANOVA, F=5.50, $p<0.0005$): most significantly between saline and Hyloprompt (Scheffe, $p<0.005$). Over fifteen minutes, the most significant differences are between Vital Eyes and the remaining drops (Scheffe, p<0.005).

There was a tendency for the magnitude of post-blink cooling to diminish (towards baseline and beyond) after five minutes for saline, Liquifilm, hypromellose, Hyloprompt, whereas for Vital Eyes this reduction appeared after 10 minutes and was more restricted. These changes were not statistically significant.

7.4 Discussion

The instillation of a single ATD into the tear system results in a complex interaction with tear film constituents and tear flow dynamics, which is still unclear from the literature. This complexity is perhaps reflected in the wide variability between subjects post-drop instillation found in this study. Large variation between normal subjects in tear film studies is not unusual, both at a clinical level and a biochemical level: Patel discussed the great inter-subject differences in tear thinning time, tear lipid and mucus levels that have been observed (Patel et al., 1988; Patel et al., 1989), and in one study using a fluorescent tracer, there was large variation between volunteers for any one ATD solution used (Ludwig et al., 1992).

It is generally agreed that the effect of aqueous tear substitutes on tear film stability does not exceed 60min to 2 hrs at most (Geeting and Bakar, 1980), and this exceeds the physical presence of the instilled fluid which is about 15-20min (Bron, 1985): the clinical impression is that some effects of ATDs last much longer than the few minutes reported in most experimental studies (Lemp et al., 1975). More recently, a study observed a reduction in ocular aberrations for dry eye patients after instillation of ATDs that was maintained to 10 minutes (Montes-Mico et al., 2004).

The results show that OST increases when artificial tears are instilled. At first this appears surprising given the temperature of the drops themselves and given that normal tear volume is 7-10µl (Korb et al., 2002), an average drop of 50µl has the ability to displace most natural fluid (Burstein, 1985). However, although their instantaneous effect will be to cool the tear film, the thermo-camera here was recording the temperature changes of the fresh tears produced 10s after instillation of the drops: when a drop is added the excess volume drains from the eye and the remaining is diluted by basal tearing and whatever reflex tearing is elicited by the instillation of the drop. The OST measured is likely to reflect the ocular response to the drops, rather than the temperature of the drops themselves. Aqueous artificial tears have been shown to cause an uneven thickening of the pre-corneal tear film, mostly superiorly (Shimmura et al., 1998): this thickening will actually be a combination of the reflex tears to the tear film as well as the drops themselves. Any irritating property of the ATD will increase lacrimation: for example, several studies have shown discomfort to arise if tear pH and the pH of solutions differ significantly (Hill, 1987). The presence of benzalkonium chloride (BAK) in Liquifilm, hypromellose and Vital Eyes might explain their 'irritant' effect on OST, but even Hyloprompt and saline (with no preservatives) still produced an initial increase in OST. Saline has been observed to *increase* symptoms in dry eye subjects (Milazzo et al., 2002): individuals will vary in the tonicity and pH of their tear film and therefore, their reaction to different formulations. Sodium Hyaluronate (0.1%) has been shown to give significantly more relief from burning symptoms compared to PVA (1.4%) (McDonald et al., 2002), and compared to saline (Condon et al., 1999): in this study there was evidence of Hyloprompt having significantly less effect on OST over time than saline. Viscosity of the different drops was not measured in this study, but it has been stated previously that the viscosity seems to have little effect on retention time and that rate of tear secretion is the primary determinant (Adler et al., 1971).

The observed decrease in OST following a blink has been previously suggested as a measure of tear film stability (Craig et al., 2000). The magnitude of post-blink cooling increased on application of all the ATDs in this study, but after 5 minutes four of them demonstrated less post-blink cooling than pre-drop recordings (Figure 7.7). This may suggest a more stable tear film exists after 5 minutes for those drops. Interestingly, it has been expressed previously (Trees and Tomlinson, 1990) that the ideal response to an ATD would be small, short-term surge in tear film evaporation rate, followed by the recovery or maintenance of a level of evaporation at or below baseline level. The reason for this increased tear film temperature that lasts longer, may be that, perversely, eyelid temperature significantly influences the delivery of meibomian gland secretion due to change in viscosity (Nagymihalyi et al., 2004), and this in turn will encourage the formulation of a more stable tear film (Watanabe, 2002). This may explain why the literature shows the physical retention times to be much less than clinical effect times of ATDs - they are washed away by the basal and reflex tearing, but it might be this tearing that encourages a more stable tear film. However, it is acknowledged that this study used normal subjects, and that dry eye subjects may react quite differently. Dry eyes may respond to the drops in a more 'positive' fashion.

The initial increase in OST seen in this study after instillation of ATDs appears at odds with the work of Morgan (1994): he noted an initial decrease at 10s followed by an increase above baseline after 10 minutes. However, as well as the use of dry eye subjects, non-preserved drops were used: this may explain the differences to this study.

7.5 Conclusions

The use of infrared thermography in this study appeared to be monitoring the lacrimal response to artificial tear drops, and it may be this response that influences the stability of the tear film post-drop instillation, rather than the active ingredients of the drops themselves, in normal subjects.

A better understanding of the ocular reaction to the application artificial tear formulations and the reduction of adverse responses with such therapies may result from critical use of this new technique.

CHAPTER 8

SUMMARY AND CONCLUSIONS

8.1 Summary

Early last century the ability to measure ocular surface temperature (OST) was recognised as being important to understanding ocular physiology, and subsequently, measurement of eye temperature has been an example of a technology-driven area of research. This thesis reviewed the disadvantages of contact methods of ocular thermometry and the evolution of infrared-imaging in ocular thermometry. Previous applications of ocular thermography have been limited by the prevailing technology: the use of scanning systems limited temporal resolution, and the use of cooled detector substances limited portability and increased expense. The desire to measure and record OST changes in real-time was identified.

In this thesis, the technique of dynamic ocular thermography using the TH7102MX thermo-camera (NEC San-ei, Japan) was examined and a suitable experimental protocol established. Procedures for collection of data and subsequent analysis were developed: the video output from the camera was evaluated in real-time (30Hz) using Labview® programming, and the large amount of data further manipulated within Excel spreadsheets. Repeatability was found to be acceptable as long as the first three measures were averaged, but limited reproducibility between sessions was recognised.

The source of the thermal radiation from the anterior eye has been subject to a degree of supposition: Hamano's work suggests that 80% of the infrared radiation from the cornea will be absorbed by a tear film thickness of 10μm (Hamano et al, 1969), and as the tear film thickness decreases the cornea will have increasing contribution. Chapter 3 of this thesis sought to further establish the theory that the tear film and its dynamic properties are the major influences on OST. It appeared that lower OST and smaller post-blink changes in temperature occurred in the presence of a more stable tear film. Other parameters (anterior chamber depth, corneal thickness and bulbar hyperaemia) appeared to have little relationship to OST).

The relationship between dynamic changes in OST and tear flow dynamics was examined in Chapter 4. This was the largest study undertaken on OST to date, and included an examination of mean values for initial OST (0.2s after a blink) and the amount and rate of post-blink cooling in normal subjects. Average initial OST was found to be 36.7±1.3ºC (centrally), and the characteristic thermal profile across the anterior eye found in other studies was observed: it became more pronounced over time post-blink, due to a faster rate of cooling centrally compared to nasally and temporally. The results from this study suggested a complex role for tear fluid dynamics in the temperature changes observed over the ocular surface post-blink, not just ocular anatomy. As the tear film thins and disperses, the underlying anatomy will have a greater contribution to the thermal profile, but because the evidence for tear film thickness is still under debate in the literature, the exact contributions of the tear film and the cornea remain unknown. These findings add further insight into the source of OST and builds on previous models (Morgan, 1994). This large study also demonstrated that the magnitude and rate of post-blink cooling was greater for females than males: possibly related to differences in tear-film properties and/or corneal curvature.

The remainder of this thesis examined the effect of contact lens wear and artificial tear drops on OST. The model eye work (Chapter 5) demonstrated the insulating effect of different hydrogel contact lenses, and a small study of neophyte contact lens wearers suggested that heat flux through the contact lenses occurred after 30-60 minutes. Observed increases in radiated temperature upon lens removal appeared to support the theory about the existence of a temperature gradient between the post-lens and pre-lens tear film due to the insulating properties of the contact lens. In Chapter 6 these ideas were further explored in the first large study using adapted wearers who had worn lenses for at least two hours, or overnight in the case of continuous wear, and comparing these with a control group. Significantly greater OST was observed in contact lens wearers, particularly with silicone hydrogel lenses compared to etafilcon A, and tended to be greatest when lenses had been worn continuously. It was suggested here that the increased OST seen with silicone hydrogel wearers may be due to the increased stiffness of these materials, affecting OST in two possible ways: increased tarsal hyperaemia (warmer tears), and less tear exchange between pre-lens and post-lens tear film. For etafilcon A lenses, heat exchange will be enhanced by increased water content and tear mixing, thereby reducing the potential for thermal build-up behind the lens. Post-blink cooling was greater and faster with lenses in situ, further establishing ideas about pre-lens tear thinning compared to pre-corneal tear film thinning. Ultimately, the finding of increased temperature behind hydrogel contact lenses may have most relevance to understanding ocular physiological responses to contact lens wear, particularly in closed eye conditions.

The effect of artificial tear drops on the tear film has previously been measured in various subjective and objective ways: Chapter 7 utilised the non-invasive properties of dynamic ocular thermography to examine their effects on OST in normal eyes in real-time. The results suggested that the thermo-camera was monitoring the ocular response to these drops, rather than the instilled temperature of the drops themselves. The biocompatibility of the solutions was observed as an influence on reflex tearing, and therefore, recorded OST. Of the artificial tear preparations examined, Hyloprompt appeared to have least 'irritating' effect on this group of normals. The technique of dynamic thermography, therefore, offered a unique measure of the interaction of the artificial tear drop with the ocular surface, and perhaps a reminder that the tonicity, pH and preservatives in a solution should be given as much attention as the 'active ingredient' in artificial tear preparations.

8.2 Conclusions and future applications

In conclusion, this thesis has shown the ability of dynamic thermography to quantify changes in the ocular surface temperature in real-time. The ability to observe and quantify post-blink changes in particular, has contributed to understanding the origins of OST, and how OST fits into existing knowledge about ocular physiology. The finding that OST increases when contact lenses are worn is important, both to *in vivo* and *in vitro* studies: understanding the ocular response to contact lens wear will remain a vast area for research. The ability of dynamic thermography to reflect the ocular response to the application of artificial tear drops may also prove to be a significant research and clinical tool.

In the future, an important development will be the ability to view the ocular surface whilst simultaneously recording thermographic data. This desire has been expressed previously (Morgan, 1994) in order to aid anatomical localisation. However, it would serve a greater purpose: the ability to simultaneously monitor thermography and tear film thinning/ flow, and possibly even thickness, using interferometric methods would be hugely beneficial, particularly in subjects with dry eye disease. Ocular thermography shares the positive attributes of many non-invasive techniques, but it is the dynamic element used in this thesis that has given most insight into the often elusive area of tears across the ocular surface. As the technology develops, its application is widening, and as a result, the price of thermal imaging equipment is decreasing. Therefore, the clinical use of a slit-lamp mounted thermography device in the future is not beyond the realms of possibility.

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APPENDIX

Supporting publications:

Purslow C, Naroo SA, Wolffsohn, JS (2004). *The Dynamic Effects Of Artificial Tear Substitutes On Ocular Surface Temperature.* The Ocular Surface: 3(1); S103.

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