Simulation of heat exposure and damage to the eye lens in a neighborhood bakery

Naomi Sharon a, Pinhas Z. Bar-Yoseph b, Elvira Bormusova a, Ahuva Dovrat a,∗

a Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel
b Computational Mechanics Laboratory, Faculty of Mechanical Engineering, Technion – Israel Institute of Technology, Haifa, Israel

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ABSTRACT
Epidemiological studies indicated a link between high temperature environment and cataract. The purpose of the study was to investigate if the high temperature in neighborhood bakeries can cause damage to the eye lens. Measurements were done to determine the temperature and exposure time in the neighborhood bakeries during a workday. Thermal analysis was done using finite volume and finite element Computational Fluid Dynamics (CFD) codes in order to determine the temperature in the eye lens when exposed to environmental temperature fluctuations. A simulation of heat exposure was carried out using a bovine lens organ culture system. Two-hundred and seventy bovine lenses were divided into five groups. (1) Control group kept in culture for 11–14 days (2) Lenses exposed to 39.5 °C, 6 h daily starting on the second day of the culture and kept in culture for 13 days (3) Lenses exposed to 39.5 °C, 4 h daily starting on the second day of the culture and kept in culture for 11 days (4) Lenses exposed to 39.5 °C, 2 h daily for 3 days starting on the second day of the culture and kept in culture for 12 days (5) Lenses exposed to 39.5 °C, 1 h on the second day of the culture and kept in culture for 14 days. Lens optical quality was assessed during the culture period. At the end of the culture lens damage was demonstrated by inverted microscopy. Lens epithelial samples were taken for analysis of Catalase activities. Control lenses maintained their optical quality throughout the 14 days of the culture. Exposure to heat caused optical damage to the cultured lenses. The damage appeared earlier in the 6 h exposure group and progressed from the lens anterior suture to its center. Optical damage was recovered in lenses exposed 1 h to 39.5 °C, but the damage remained in the lens epithelial cells. Our study indicates that exposure to heat in bakeries can cause damage to the eye lens and that the damage is dependent on the length of exposure.

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1. Introduction

Epidemiological and clinical observations have indicated a link between heat exposure and cataract. Workers in the glass industry, which involves high environmental temperatures, are at 2.5 higher risk to lose 30% of their sight as a result of cataract, compared with people from the same group of age who are not exposed to high temperature stress (Lydhal and Philipson, 1984). There has been evidence for cataract formation following exposure to infra-red radiation, radiation emitted from hot material (Okuno, 1990). It was reported that workers in the molten metal industry are at higher risk for cataract formation (Lankatilake and de Fonseka, 1990). It was not indicated what regulation regarding temperatures and time exposure is recommended.

There is also a lack of information regarding the temperatures that eventually reach the eye lens in a high temperature environment such as a bakery. In the present study we followed the thermal effects on the lens and the damage it causes as a result of heat exposure in a neighborhood bakery after we measured and calculated the temperature distribution in the eye.

The originality of this work is the study of the effects of heat on the intact lens in long-term culture conditions, and following the ability of the intact lens to recover form heat damage, in contrast to the effects of heat on isolated proteins from the eye lens (Horwitz, 1992; Horwitz et al., 1992; Borkman and McLaughlin, 1995).

2. Materials and methods

2.1. Determination of temperatures and exposure time in the neighborhood bakery

In order to simulate the conditions of bakery workers subjected to high environmental temperatures in their work place, the
temperatures and exposure time were measured in a bakery during six working days. In the bakery, workers are exposed to high temperature when working close to the electrical oven. The workers lack any eye protection. As part of the daily work, workers push their heads into the electrical oven. The measurements were taken by attaching a thermometer probe to the temporal side of the eyeball of the worker, and following the temperature changes during a working day.

2.2. Thermal analysis of the heat reaching the eye lens

A finite element simulation of the bio-heat transfer equation in the human eye was first conducted by Scott (1988a,b). This method was used to determine the temperature in the human eye induced by infra-red radiation (Scott, 1988a,b). Later on, it was used by Okuno (1990, 1993) to study the thermal effects of visible light and infrared radiation. In the present study Galerkin finite element formulation and conservative finite volume scheme (Fluent CFD software) were used to resolve the bioheat transfer problem, Eqs. (1)–(4), and to predict the conductive heat transfer in steady-state, and the history of the temperature distribution in the lens as a function of changes in surface conditions and time.

The eye is modeled as shown in Fig. 1A; dimensions were taken from Scott (1988a). A typical unstructured mesh consists of 5500 elements. The mesh is gradually refined towards the cornea, where most of the heat transfer takes place.

The model allows predicting the history of the temperature distribution in the human eye as a result of the cornea being transiently exposed to different types of external boundary conditions (mainly temperature).

2.3. Simulation of heat exposure using a lens organ culture system

Based on the thermal analysis results, a simulation in the laboratory was done using bovine lenses in an organ culture system. Bovine lenses were carefully excised from eyes obtained from animals up to 1-year-old, 2–4 h after enucleation. Each lens was placed in a specially designed culture cell with two compartments (Dovrat et al., 1986). Both lens surfaces were bathed in the culture medium (24 ml) consisting of M199 with Earle’s salts, antibiotics (Penicillin 100 U/ml and Streptomycin 0.1 mg/ml) and 3% fetal bovine serum. All lenses were incubated at 35 °C. Experimental treatments were initiated after pre-incubation of 24 h. Only non-damaged lenses were included in the study.

2.4. Lens optical quality monitoring

The optical quality of the lenses was analyzed each day of the culture using a low power laser scanner. The scanner consists of a 2 mW, 670 nm helium-neon diode laser mounted on a computer-controlled X-Y table, and a television camera with a video frame digitizer (Sivak et al., 1990). The laser beam was parallel to the axis of the lens and was directed towards the cultured lens along one meridian in 0.5 mm increments. After passing through the lens, the laser beam is refracted and the system determines the back vertex focal length for every beam position. Each scan consists of measurements of the same beam from 22 different points across the lens. A lens of good optical quality is able to focus the laser beam from the various locations. When the lens is damaged its ability to focus the laser beam at the various locations is altered.

2.5. Lens photography using inverted microscopy

Photographs of five lenses from each treatment group and their contralateral control eye lenses were performed using an inverted microscope. We took pictures of the lenses periphery and centers using a final magnification of ×25 and ×100.

2.6. Analysis of catalase activities in lens epithelium

Lens epithelium was dissected under a binocular stereomicroscope. The lens capsule and its adherent epithelium were removed from the entire lens. The tissue was immersed immediately in a 200-µl volume of 50 mM phosphate buffer, pH 7.0. All further work was carried out at 0–4 °C. The tissue was sonicated in an MSE 150 W ultrasonic disintegrator (MSE; UK) at 50 W for 10 s twice followed by centrifugation at 14,000g for 10 min. Catalase activities of the supernatant were measured. Catalase activity was measured according to the method of Beers and Sizer (1952) by spectrophotometer recording of the cleavage of H₂O₂ at 240 nm.
The total time of exposure to 39.5 °C, when the door was open the temperature was 58 °C, workers are continually exposed to heat. In the test case corresponding to the work cycle in a bakery, intact bovine lenses were incubated in culture for about 2 weeks. Control lenses were incubated at 35 °C throughout incubation period. Heat treated lenses were exposed to 39.5 °C for different times. In our simulation process we followed the gradient of Fig. 1D by starting the exposure to heat with culture medium from the anterior part of the lens (epithelium) to the posterior part of the lens. The total time of exposure to 39.5 °C was approximately 6 h out of an 8 h working day, while the time which a worker spends in the 39.5 °C continually was approximately 4 h.

In order to simulate the working conditions in the neighborhood bakery, intact bovine lenses were incubated in culture for about 2 weeks. Control lenses were incubated at 35 °C throughout incubation period. Heat treated lenses were exposed to 39.5 °C for different times. In our simulation process we followed the gradient of Fig. 1D by starting the exposure to heat with culture medium heated to 39.5 °C in the anterior compartments of the culture cells (Dovrat et al., 1986) and then incubated the culture cells in an incubator at 39.5 °C for the period of the analysis. In the initial experiments, lenses were exposed to 39.5 °C for 6 h a day, (Fig. 2A) 5 days a week, while simulating the maximum time exposure during each working day. In the following experiments lenses were exposed to 39.5 °C, 4 h a day (Fig. 2B) simulating the time that the workers are continually exposed to heat. Fig. 2A demonstrates the optical damage that appeared in lenses exposed to 39.5 °C, 6 h a day for 4 or 8 days. Focal length variability represented the variation in the focal lengths of the 22 beams that passed through the lens during each scan and was calculated as the standard error of the mean (SEM) of the 22 focal lengths. There was almost no change in focal length variability (ability of lenses to focus light) in the control lenses during the culture period. Lenses exposed repetitively to 39.5 °C 6 h a day for 8 days showed an increase in focal length variability (optical damage) after 6 days in culture (Fig. 2A). Focal length variability, increased up to two-fold after 6 days and up to 33 mm on day 13 of the culture. These changes did not return to control values during the experimental period. Large variations in the results indicate that different lenses react differently. Some lenses were more damaged than others.

Lenses exposed repetitively to 39.5 °C, 6 h a day for 4 days showed less damage between days 7 and 8, 2 days after the last exposure to

### Table 1

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<td>Environmental temperatures</td>
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<td>Lens temperatures</td>
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Fluctuations in the eye lens temperatures as a function of the temperature in the bakery during winter time and during summer time. The temperature inside the bakery distant from the oven was 23 °C in winter and 36 °C in summer. In front of the oven, when the door was open the temperature was 58 °C. Lens temperatures were obtained from the thermal analysis model using the fluent software.

2.7. Statistical analysis

All results were analyzed using Student’s paired t-test. A change was defined as significant if the difference between control and treated groups reached $P < 0.05$. 

3. Results

In the test case corresponding to the work cycle in a bakery during a winter day, the initial temperature of the work cycle is 23 °C (Table 1). Temperatures fluctuate between 23 and 58 °C at various time intervals (Fig. 1B). The temperature that eventually reached the lens fluctuated between 35 and 39.5 °C (Fig. 1C); the temperature changes were in the form of a staircase. The temperature distribution over the eyeball in a given time interval is demonstrated in Fig. 1D which constantly showed a gradient of 1.5 °C from the anterior part of the lens (epithelium) to the posterior part of the lens. The total time of exposure to 39.5 °C was approximately 6 h out of an 8 h working day, while the time which a worker spends in the 39.5 °C continually was approximately 4 h.
At this point the lenses were able to repair the optical damage in between when the frequency of exposures was decreased from 8 times to 4 times, and return to control values temporarily, hence eventually permanent damage was visible on day 9–13. The damage increased to the same values as for the longer exposure to heat on day 12 with no further increase on day 13.

The optical damage was smaller when the exposure time to heat was reduced as shown in Fig. 2B. Lenses exposed to 39.5 °C, 4 h a day showed less optical damage than lenses exposed to heat 6 h a day for 4 days or 8 days. In that case the two-fold increase in focal length variability appeared on day 8 of the culture, and increased up to 15 mm on day 10 of the culture, when the lenses were exposed 8 times. Lenses exposed to heat 4 h, 4 times showed less damage than lenses exposed 8 times. Focal length variability, increased up to two-fold after 10 days in culture with no repair during the culture period.

Fig. 2C illustrates an attempt to determine a threshold regarding exposure frequency and duration, with lenses exposed to 39.5 °C. The exposure time was reduced to 2 h in order to try and find the threshold where no permanent damage to the eye lens will occur. In that case the focal length variability, increased up to two-fold on day 10 of the culture with no further increase in the damage.

Since there was permanent damage in this group the next step was to evaluate the damage of a single exposure to 39.5 °C for 1 h on the second day of the experiment Fig. 2D. Treated lenses did not reach the two-fold focal length variability. Lenses showed fluctuating focal variability no greater than 8 mm with the ability to repair the damage and return to the base value at the end of the experiment.

On gross examination with the unaided eye, lenses exposed to heat for 1 h, 2 h and some of the lenses exposed to heat for 4 h, were clear at the end of the culture period. On the other hand, most of the lenses exposed to 6 h heat show separation of the sutures that could be seen with the unaided eye. At the end of the culture period, lenses were photographed using an inverted microscope and the lens epithelium was analyzed for catalase activities.

Fig. 3A demonstrates inverted microscopy of control undamaged lens with intact epithelial layer and heat-treated lens of 39.5 °C, 4 h, 4 times with vacuoles spread all over the lens. Photographs of the cortical area (Fig. 3B) show control lens with intact...
fiber cells and heat-treated lens of 39.5 °C, 2 h, 3 times with vacuoles between the fiber cells. Reduction of the heat exposure to 1 h, 1 time did not prevent the cell damage. Although the optical damage was repaired the damage remained in the lens cells. Fig. 3C shows inverted microscopy on day 15 of the culture of control lens with intact fibers of the cortex area and heat-treated lens (39.5 °C, 1 h, 1 time) on day 15 of the culture (microscope magnification ×100). On the left, undamaged control lens with intact fibers of the cortex area. On the right heat-treated lens with blurred appearance of the fibers, indicating fluid accumulation.

Fig. 4 shows catalase activities in lens epithelium on day 11 of the culture after different heat exposures. Control lenses show activities of about 3.5 U/mg protein after 11 days in culture. Lenses exposed to 39.5 °C for 1 h on day 2 of the culture and kept in culture for 11 days show increased catalase activities to about 7 U/mg protein. Increase in heat treatment to 2 h on days 2, 3 and 4 of the culture and measurement of the activities on day 11 show increased activities up to 18 U/mg protein. From that point of heat incubation, increasing the time of exposure to heat of 39.5 °C reduced catalase activities on day 11. When lenses were exposed to heat 6 h a day for 8 days, catalase activity on day 11 of the culture was almost zero.

4. Discussion

From our measurements of the ambient temperatures in the neighborhood bakery, and on the basis of thermal analysis of the actual heat reaching the human lenses, we conclude that lenses are exposed to high temperatures and to large temperature fluctuations in the neighborhood bakery. This effect may account for the working conditions of the bakery workers, who spend up to 6 h per day near electric ovens. The lack of use of protective eyewear that isolate the eye from the heat (for instance glasses with a gold mirror coating which reflects 90% of the radiated heat) probably exposes bakery workers' lenses to excessive heat. Sasaki et al. (2002) conducted cataract epidemiological studies in four different places Noto and Amami in Japan, Reykjavik in Iceland, and Singapore. The
evaluation and grading of lens opacities were done using graphic analysis of Scheimpflug and retro-illumination images. They found prevalence of nuclear opacity extremely high in Singapore, followed by Amami. The main type of lens opacity was nuclear in the Singapore group and cortical in the Noto and Icelandic groups. Lens opacity in Amami was intermediate between the above two groups. They connected the high prevalence of nuclear opacity with high UV exposure and high ambient temperature.

Kojima et al. (2002) conducted a long-term study with rats by exposing Brown Norway rats to 35 °C or 24 °C. Each group was divided to streptozotocin-induced diabetes, ultraviolet B exposure and controls. Three weeks after the start of the experiment the high temperature controls showed higher light scattering by Slit-lamp microscopy and an anterior image analysis system than that of the controls exposed to 24 °C. An increase in abnormal nuclear scattering light in the crystalline lens of this group was found 9 weeks after the start of the experiment, and at the end of the experiment (78 weeks later) dense abnormal nuclear light scattering was found including the pre-nuclear area. This study shows that long-term exposure to heat causes nuclear cataract. From our study, we are unable to determine if people who work in these bakeries suffer from cataract because usually young people are employed in this job for a limited time of a few months before starting their studies. We believe our lens culture system (because of limited repair mechanisms) can simulate in a relatively short time of 14 days, the damage which appears in the intact body after long-term exposure to heat.

When we exposed bovine lenses in culture conditions to the same temperature and time that the human lenses were exposed in the bakery during a working week, 4 and 6 h of 39.5 °C for 8 days, optical damage was demonstrated by recording higher focal length variability values in comparison to control lenses, which were kept at 35 °C. The longer time the exposure to heat, the greater the damage appeared in lens optical quality (dose–response manner). Photography carried out during the incubation period (data not shown) showed lens damage on the same days of the culture and kept in culture conditions for 11 days. The exposure to heat was with 24 h intervals.

Fig. 4. Catalase activity measurements (U/mg protein) in lens epithelium on day 11 of the culture period. Control lenses were kept in culture at 35 °C for 11 days. Heat treated lenses were exposed to 39.5 °C start on the second day of the culture and kept in culture conditions for 11 days. The exposure to heat was with 24 h intervals.

When we reduced the time of exposure of the lenses to heat up to 2 h of 39.5 °C for 4 days only, we were able to deduce that lens damage is influenced both by the exposure length (4 h versus 6 h) and by the frequency of exposures (8 times versus 4 times).

Reducing the time of exposure to heat to 2 h of 39.5 °C for 3 days also showed optical and morphological damage but the damage was less prominent than the damage from 4 and 6 h of exposure. Lenses exposed in culture to 39.5 °C for only 1 h on the second day of the culture could repair the optical damage, but morphologically there were changes that indicate irreversible damage to the epithelium and fiber cells.

Catalase activities measurements on day 11 of the culture period showed increased activities of the enzyme as a result of heat exposure up to 2 h exposure to 39.5 °C for 3 times. We are still unable to determine if the increasing activity is a result of activation of catalase molecules or new synthesis of enzyme molecules as a result of the heat stress.

Increasing the heat exposure time to 4 h, 4 times and more reduced the enzyme activities. When lenses in culture were exposed to heat 4 h, 8 times catalase activities were below control levels which means damage to the enzyme molecules without the ability to recover in culture conditions. Catalase is very sensitive to UV because of the increase of free radicals and H2O2 in the lens as a result of UV exposure (Azzam and Dovrat, 2004). The effect of heat on catalase was demonstrated also by Chiang and Chou (2008). Both heat shock of 42 °C and ethanol shock reduced the levels of SOD and catalase activities in Vibrio parahaemolyticus. Our study shows an increase of catalase activities a few days after short exposure to 39.5 °C and decreased catalase activities after longer exposures to 39.5 °C.

Catalase activities were almost zero on day 11 after lenses in culture were exposed to heat of 6 h, 8 times, the same conditions as bakery workers have during their work days. We assume that the repair mechanisms in the intact body is much better than in culture conditions, but zero activity means no protection of the enzyme from oxidative insults.

Heys et al. (2007) exposed intact pig lenses to elevated temperatures (50 °C) and found the same protein changes associated with a progressive increase in lens stiffness as in presbyopia. They suggested that presbyopia may be the result of a loss of alpha-crystallins coupled with progressive heat-induced denaturation of structural proteins in the lens during the first five decades of human life. The protein integrity, which is essential for cellular homeostasis, is maintained by a complex system of refolding or degradation of damaged proteins. The heat shock proteins are the major contributors to the maintenance of protein integrity. Cells must be able to respond rapidly to changes in their environment in order to maintain homeostasis and survive. Induction of heat shock proteins is a common cellular defense mechanism in response to various stress stimuli. Heat shock factors (HSF) are transcriptional regulators which function as molecular chaperones in protecting cells against damage. Mammals have three functionally distinct HSFs: HSF1 is essential for the heat shock response and is also required for developmental processes, whereas HSF2 and HSF4 are important for differentiation and development. Specifically, HSF2 is involved in corticogenesis and spermatogenesis, and HSF4 is needed for maintenance of sensory organs, such as the lens and the olfactory epithelium. Akerfelt et al. (2007) showed the different roles of the mammalian HSFs as regulators of cellular stress and developmental processes. They suggested a functional interplay between HSF1 and HSF2 in the regulation of Hsp expression under stress conditions. In lens formation, HSF1 and HSF4 have been shown to have opposite effects on gene expression. Yao et al. (2006) investigated the dynamic expression of heat shock protein (Hsp) 70 and Hsp 27 in lens epithelial cells of contused eyes and the effects of heat shock and quercetin. Preconditioning hyperthermia (45 °C, 8 min) resulted in a significant increase of Hsp70 expression. Increased expression of Hsp70 in lens epithelial cells of contused eyes may play a protective role against degeneration of lens proteins. Thermal preconditioning possibly protects against lens injury by increasing the expression of Hsp70. Endogenous protective mechanisms may be important in eye contusion. Sun and MacRae (2005)
explored the involvement of sHSPs in disease and their potential for therapeutic intervention. They suggested that as molecular chaperones, the sHSPs protect protein structure and activity, thereby preventing disease, but they may contribute to cell malfunction when perturbed. For example, sHSPs prevent cataract in the mammalian lens and guard against ischemic and reperfusion injury due to heart attack and stroke. On the other hand, mutated sHSPs are implicated in diseases such as desmin-related myopathy and they have a possible relationship to neurological disorders including Parkinson’s and Alzheimer’s disease.

In the present study we demonstrated that high ambient temperatures cause optical and structural damage to bovine lenses. The longer the time and the greater the frequency of exposure, the greater also was the damage shown by the cultured lenses. A particularly interesting finding was that even a short time of exposure to high temperature caused optical damage to cultured lenses, but the lenses were able to overcome this damage by self-repair mechanisms. Although one should be careful in extrapolating data from isolated lenses in organ culture to human beings, our study may nevertheless indicate a need for the use of eyewear protection in bakeries. On the other hand, the issues of temperature’s impact on lens clarity and the involvement of heat exposure in cataract formation require further investigation.

Successful use of kinetic models of thermal damage processes depends critically on the identification of a quantitative measure of thermal damage. Kinetic models based upon the Arrhenius damage integral are frequently used in order to characterize the combined effects of temperature and exposure time on protein denaturation (Kampmeier et al., 2000). The temperature calculations can be used in a single Arrhenius rate process to quantify the accumulation of thermal damage mechanisms in the lens. This issue is worth further investigation and will be considered as a possible future extension of our present study.

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