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POSSIBILITY OF INCREASE IN RISK OF AGEING OF AN EYE LENS AT INFLUENCE OF HEATS ON WORKPLACES, SUCH AS GLASS FACTORY, A BAKERY

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Introduction

Aging of the eye lens involves reduction in enzyme activities along with accumulation of non-active partially denatured enzyme molecules. These observations were demonstrated in rat lenses [1-5] and also in human lenses [6]. Reduction in enzyme activities is accompanied with stiffness of the older section of the lens (lens nucleus), which leads to presbyopia and cataract formation. Cataract, opacity of the eye lens, is one of the major causes of loss of vision in the world. Several risk factors have been recognized as contributing to lens aging and cataract formation, including ultraviolet light, which reduces enzyme activities in human lenses [7] and also reduces enzyme activities in bovine lenses in culture conditions [8-10]. Ultraviolet light increases the activity of the cross-linking enzyme transglutaminase (TGase). TGases are calcium-dependent acyltransferases. These enzymes are widely distributed in cells and body fluids and are involved in coagulation of blood, formation of hair follicles, wound healing, cellular growth regulation, differentiation, aging [11-12] and drug-induced post-translational modification of proteins [13]. TGases are also connected to cross-linking of lens crystallins [14-15]. TGase activity in the lens was affected by UVA radiation [16]. The activity of TGase in lens epithelium cortex and nucleus increased as a result of the UVA irradiation and then declined towards control levels during the culture period, as the lens recovered from the UVA damage. Specific lens proteins, B and B1 crystallins (the enzyme substrates) were affected as shown by the appearance of aggregation and degradation products. It appears that TGase is involved in the mechanism by which UVA causes damage to the eye lens. UVA also cause changes in lens morphology [17-18] plasma membrane and cytoskeletal lens proteins [19-20] and alpha crystalline chaperon activity [21]. Another risk factor is diabetes. Diabetic cataracts are one of the severe complications appearing in diabetes. Diabetics are prone to develop cataracts at much younger ages than healthy people in their age group. Many studies have shown an association of lens opacities and cataract with diabetes. Hiller and Kahn[22] reported that diabetes substantially increases the probability of cataract extraction in the age group from 40 to 49, and approximately doubles or triples this probability for ages 50 to 69. Ederer et al. [23] found that diabetic subjects between the age of 55 and 64 years have a three-fold greater risk of developing cataract than non-diabetics. There are several theories on the possible mechanisms leading to diabetic cataract. Most of the studies indicate that hyperglycemia is the major cause for diabetic cataract development. 1) Increase in glucose concentrations in the eye lens increase the activity of aldose reductase (AR) [24]. This enzyme, which is induced by high glucose concentrations, reduces glucose to sorbitol by using the reduction power of NADPH. Sorbitol cannot leave the lens, causing an osmotic effect which causes a

mass influx of water, swelling of the cells, and to a series of deleterious effects in the lens, leading to cataract formation. 2) High glucose concentrations increase lens metabolism and oxidative stress. According to this theory, oxidative stress is the major cause for cataract. 3) Hyperglycemia causes an increase in glycation of lens proteins [25]. Creation of cross-linking between groups in the proteins causes protein aggregates and reduces light transfer through the lens [26]. In addition, glycated compounds are more sensitive to oxidation. Epidemiological studies have indicated a link between high environmental temperature and cataract [27] It was found that the prevalence of nuclear opacity is extremely high in Singapore. The high prevalence of nuclear opacity was connected with high UV exposure and high ambient temperature. In addition to high ambient temperatures there are workplaces with excessively high temperatures such as bakeries and glass factories. In order to simulate the conditions of workers subjected to high environmental temperatures in their workplace, the temperatures and exposure time were measured during 6 working days. In the bakery, 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. A finite element simulation of the bio-heat transfer equation in the human eye was first conducted by Scott in 1988 [28]. This method was used to determine the temperature in the human eye induced by infrared radiation. Later on it was used by Okuno [29-30] to study the thermal effects of visible light and infrared radiation. In our previous study [31] Galerkin finite element formulation and conservative finite volume scheme were used to solve the bio-heat transfer equation predicting the conductive heat transfer in steady-state and the history of the temperature distribution in the lens as a function of surface changes conditions over time. We used this information to simulate the heat conditions at two workplaces using a bovine lens organ culture system. Our culture system is a unique and powerful tool which can help in understanding the mechanisms of cataract formation resulting from different insult factors. Our system permits exposure of intact cultured lenses to controlled specific factors and subsequently following the developing stages of the damage. The system mimics the lens conditions inside the eye and makes it possible to keep lenses for long-term studies in order to test the effects of potentially damaging and protecting agents. The high sensitivity of the system permits analysis of changes in optical quality, which already appear when the lens is still transparent.

Methods

Lens organ culture system

Lenses were excised from eyes obtained from 1-year-old male calves, 2-4 hours after enucleation. Each lens was placed in a culture container consisting of two compartments connected by a round hole. Lenses were completely immersed in culture medium [32]. The culture medium consisted of 24ml M199 with Earl's balanced salt solution, 3% fetal calf serum, and antibiotics (Penicillin 100 U/ml and Streptomycin 0.1 mg/ml) and was changed daily. The lenses were incubated at 35°C. Experimental treatments started after pre-incubation of 24 hours. Damaged lenses were excluded prior to experimental treatment.

Optical quality monitoring

Lens optical quality was monitored daily throughout the culture period. Lens optical measurements were determined by an automated scanning laser system that recorded focal length across the lens. The laser scanner consisted of a low-powered helium-neon laser mounted on a

computer-driven X-Y table with two video cameras and a video frame digitizer. The laser was programmed to scan across the lens in the axial direction in steps of 0.5 mm, while the video cameras transmitted the image of the refracted beam to the video digitizer. A custom software program determined the focal length of each refracted beam from the digitizer image. The optical center was first determined for each lens by finding the position of minimum refraction for both the X and Y directions. 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 [33]. Back Vertex Distance (BVD) represented the variation in the focal lengths of the 22 points passed through the lens during each scan and was calculated as the standard error of the mean (SEM) of the 22 focal lengths.

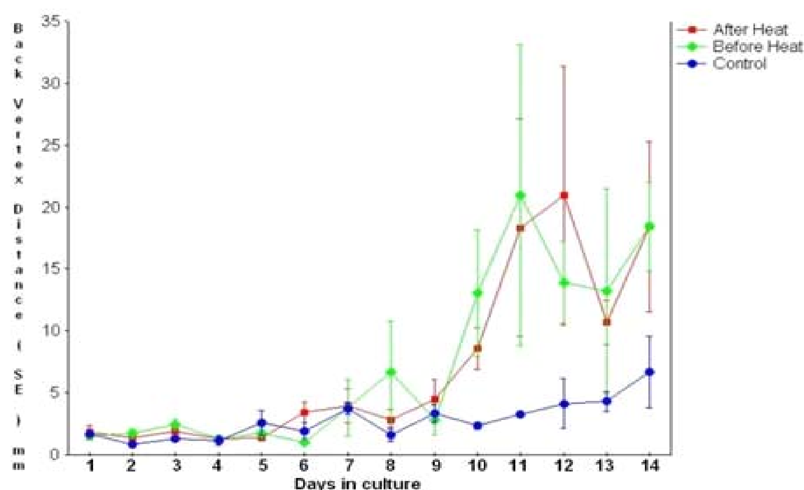

Fig. 1. Optical quality of lenses exposed to 37,8°C for 75 min each day of the culture. Control lenses show almost no variation in focal length during the 14 days of the culture period. Lenses exposed to heat show damage starting on day 10 of the culture with small recovery steps along the culture period.

Experimental groups

Four experimental groups of lenses were studied

1). Lenses exposed each day of the culture to 37.8°C for 75 min and kept in culture conditions for 14-15 days.

2). Control lenses from the contra-lateral eye of group 1 kept in culture 14-15 days.

3). Lenses exposed to 39.5°C for 2 hours 3 times with 24 hours interval between treatment starting on day 2 of the culture and kept in culture 11 days.

4). Control lenses from the contralateral eye of group 3 were kept in culture 11 days. 80 lenses were used in the study, 20 lenses for each treatment and 20 lenses for each control.

Lens epithelium histochemistry

After 2, 11 or 14-15 days in culture the lens capsule was carefully removed, and the epithelium was analyzed for ATPase activity. Histochemical analysis of magnesium-activated Na,K ATPase was performed according to Padykula, Herman[34]. Quantitative analysis was performed using Image-Pro Plus 5.0 software and is presented as the area of ATPase staining in μm2 or as the percentage of the total microscope field of view.

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 a value of $P < 0.05$.

Results

Simulation experiments of the heat reaching the eye lens Incubation of bovine lenses in culture conditions and exposes the lenses to heat of 37.8 °C each day of the culture for 75min, result in optical damage to the lenses starting on day 10 of the culture Fig. (1). On day 10 the lenses lost their ability to focus light as shown by increased variation in back vertex distance – the distance between the lens and the focal point. From day 10

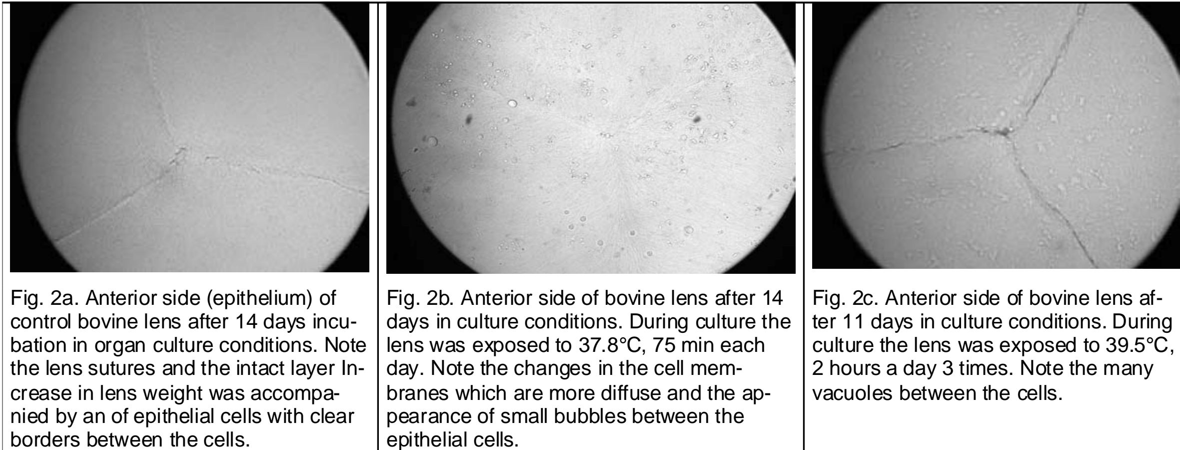

Fig. 2a. Anterior side (epithelium) of control bovine lens after 14 days incubation in organ culture conditions. Note the lens sutures and the intact layer Increase in lens weight was accompanied by an of epithelial cells with clear borders between the cells.	Fig. 2b. Anterior side of bovine lens after 14 days in culture conditions. During culture the lens was exposed to 37.8°C, 75 min each day. Note the changes in the cell membranes which are more diffuse and the appearance of small bubbles between the epithelial cells.	Fig. 2c. Anterior side of bovine lens after 11 days in culture conditions. During culture the lens was exposed to 39.5°C, 2 hours a day 3 times. Note the many vacuoles between the cells.

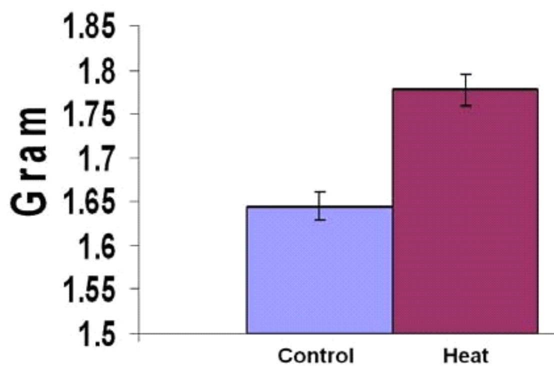

Fig. 3 Exposure to heat during culture conditions causes lens swelling which increased weight lens.

of the culture and later, the optical damage increased in the heat treated lenses. Control lenses show almost no variation in back vertex distance during the 14 days of the culture. Lenses exposed to 39.5°C also show optical damage.

Fig. 2a demonstrates a control unharmed lens incubated in culture conditions at 35°C for 14 days. The micrograph shows the anterior center of the lens with the sutures and the lens epithelium layer. The epithelial cells are intact and clear membrane borders are visualized between the cells. In contrast, a lens which was exposed to 37.8°C each day for 75min shows damage to epithelial cell membranes (Fig. 2b) and small bu5bbles appeared between the cells. The damage increased in lenses that were exposed to 39.5°C. Fig. 2c demonstrates a lens on day 11 of the culture which was exposed to 39.5°C for 2 hours on day 2, 3 and 4 of the culture period. In addition to the melting like appearance of the membrane between the cells, big vacuoles appeared between the epithelial cells and also between the lens fiber cells. This damage can suggest interference with water and ion balance in the lens.

Increased lens weight

The appearance of vacuoles in the lenses as a result of exposure to heat, indicate disruption of the cell membranes and accumulation of water inside and between the cells. Fig. 3 demonstrates the average weight of 20 control lenses incubated in culture for 14 days and 20 lenses exposed to 37.8°C each day of the culture for 75 min. The average weight of the lenses exposed to heat increased by about 130mg which is about 8% of the normal lens weight.

Fig. 4a demonstrates the increase in lens epithelial cell area after exposure of intact bovine lenses to 37.8°C 75min each day of the culture. The older section of the lens epithelium, the center of the epithelial layer was affected more than the younger section at the equators of the lens epithelium. Exposing the intact lenses to 39.5°C for 2 hours 3 times during the incubation in culture (Fig. 4b) also shows an increase in cell area, but less than exposing each day to 37.8°C. There is no difference between the older section (center) and the younger section (equators) of the lens epithelium. The exposure to39.5°C was only on 3 days of the culture day 2, 3, 4, and after that the

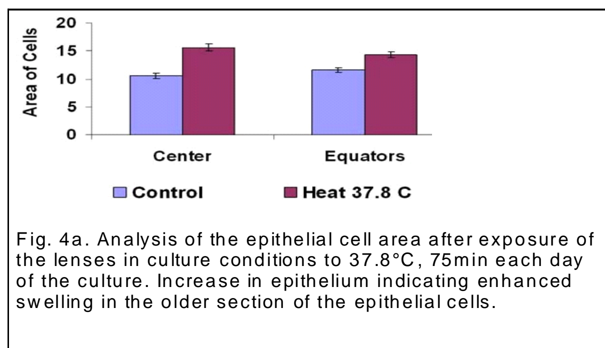

Fig. 4a. Analysis of the epithelial cell area after exposure of the lenses in culture conditions to 37.8°C, 75min each day of the culture. Increase in epithelium indicating enhanced swelling in the older section of the epithelial cells.

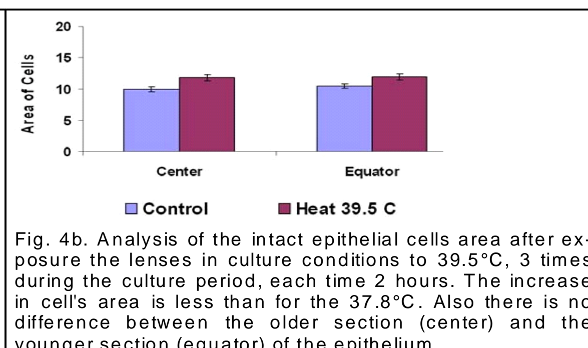

Fig. 4b. Analysis of the intact epithelial cells area after exposure the lenses in culture conditions to 39.5°C, 3 times during the culture period, each time 2 hours. The increase in cell's area is less than for the 37.8°C. Also there is no difference between the older section (center) and the younger section (equator) of the epithelium.

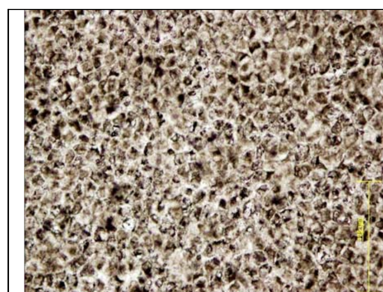

Fig. 5a. ATPase activity in control bovine lens epithelium after 14 days incubation in organ culture conditions.

Fig. 5b. ATPase activity in bovine lens epithelium after 14 days incubation in organ culture conditions and exposure 75min each day to 37.8°C. Note the increase of ATPase activity compared with control lens (Fig. 5a)

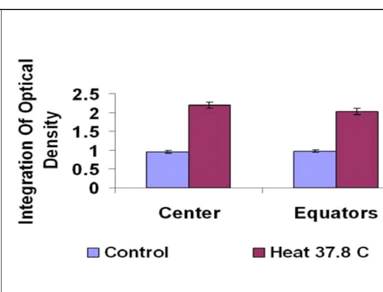

Fig. 5c. ATPase activity analysis after exposure of the lenses in culture conditions to 37.8°C, 75min each day of the culture. ATPase activities increased 2 times at the center of the lens epithelium and also increased at the equators of the lens epithelium.

lenses had time to recover from the heat damage till the end of the culture period on day 11.

Adenosine triphosphatase

The activities of adenosine triphosphatase (ATPase) were followed in order to understand the effects of heat on the membrane of the cells. This enzyme is responsible for the breakdown of ATP to ADP (adenosine di-phosphate) with the release of free energy. The enzyme also is involved in the sodium pump, which plays a key role in maintenance of ion composition in the cells using the energy derived from ATP hydrolysis to shift sodium outward and potassium inward. Exposure of lenses in culture conditions to heat of 37.8°C for 75 min each day causes an increase in ATPase activities in lens epithelium (Fig 5a, 5b).

The increased activity is despite the damage to lens epithelial membranes (Fig. 2b). Exposing the lenses to 39.5°C reduced ATPase activity immediately after the incubation at this high temperature (Fig. 6a, 6b) but with time in culture the activity is partially recovered (Fig. 6c). Lenses were exposed to 39.5°C for 2 hours on days 2, 3 and 4 of the culture and retained in culture for 11 days. It took 7 days in culture for ATPase to recover from 69% activity to 83% activity at the center (oldest section) of the lens epithelium and from 55% activity to 78% at the equators of the lens epithelium (Fig. 6d, 6e). The recovery in ATPase activity did not protect the lens from the heat damage seen in Fig. 2c.

Fig. 6e. Analysis of ATPase activity on day 11 of the culture period, after exposure of the lenses in culture conditions to 39.5°C, 3 times during the culture, each time 2 hours. ATPase activities were reduced at the center of the lens epithelium, also at the equators of the lens epithelium compared with control lenses, but there was some recovery of the activity compared with the analysis done immediately after exposure to heat Fig. 6d.

Discussion

Our study demonstrated damage to

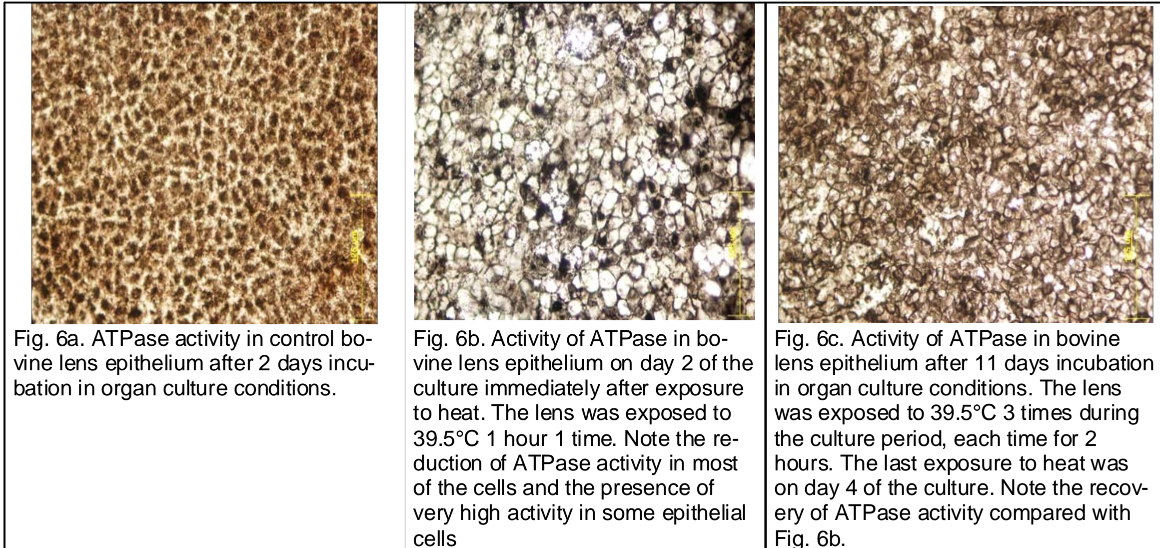

Fig. 6a. ATPase activity in control bovine lens epithelium after 2 days incubation in organ culture conditions.

Fig. 6b. Activity of ATPase in bovine lens epithelium on day 2 of the culture immediately after exposure to heat. The lens was exposed to 39.5°C 1 hour 1 time. Note the reduction of ATPase activity in most of the cells and the presence of very high activity in some epithelial cells

Fig. 6c. Activity of ATPase in bovine lens epithelium after 11 days incubation in organ culture conditions. The lens was exposed to 39.5°C 3 times during the culture period, each time for 2 hours. The last exposure to heat was on day 4 of the culture. Note the recovery of ATPase activity compared with Fig. 6b.

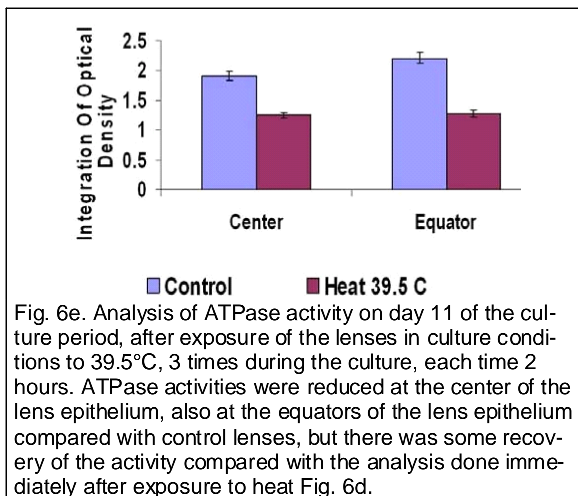

Fig. 6e. Analysis of ATPase activity on day 11 of the culture period, after exposure of the lenses in culture conditions to 39.5°C, 3 times during the culture, each time 2 hours. ATPase activities were reduced at the center of the lens epithelium, also at the equators of the lens epithelium compared with control lenses, but there was some recovery of the activity compared with the analysis done immediately after exposure to heat Fig. 6d.

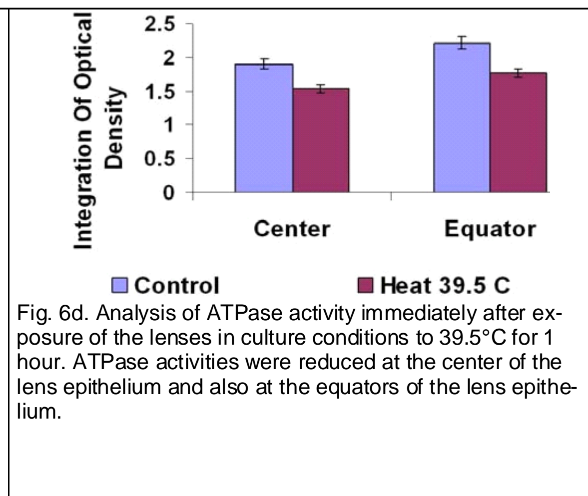

Fig. 6d. Analysis of ATPase activity immediately after exposure of the lenses in culture conditions to 39.5°C for 1 hour. ATPase activities were reduced at the center of the lens epithelium and also at the equators of the lens epithelium.

the eye lens as a response to heat similar to that in neighborhood bakeries and glass factories. The damage which can appear in humans after 10 or 20 years appears in culture within 10 to 14 days because of limited repair mechanisms in culture conditions. There is increasing evidence that heat is one of the major insults which leads to premature aging of the lens leading to presbyopia and cataract formation. Miranda [35] found a linear correlation between ambient temperature and the age of onset of presbyopia. With age the human lens loses elasticity and increases viscosity. This may account for the loss of accommodation with the development of presbyopia [36]. In our study we demonstrated that lenses exposed to heat lost their ability to focus light. In addition, lens volume increased, the membranes of the cells were modified and vacuoles appeared between the cells. Sodium-potassium-adenosine triphosphatase (Na,K-ATPase) has been recognized for its role in regulating electrolyte concentrations in the lens. The electrolyte balance is vital to keep lens water balance and lens transparency. Tseng and Tang [37] compared the abundance of the alpha-subunit of Na,K-ATPase in lens epithelia of patients with senile cataracts. Immunoblotting revealed that the amount of Na,K-ATPase alpha-subunit tended to decrease with increased cataract severity. The inverse correlation was significant only in the cortical region adjacent to the lens epithelium. It is directly affected by the loss of function of Na,K-ATPase in the epithelium. Such loss could result in water accumulation and

appearance of vesicles. Their description of the results which appear for damage to ATPase is identical to our results after exposure of the lenses to heat. Our study shows that when the stress is small (37.8°C), the lenses respond by increasing ATPase activity. Lenses in culture cannot cope with exposure to heat of 39.5 °C leading to a reduction in ATPase activity, but even so after the insult stopped on day 4 of the culture the lenses start to repair the damage and on day 11 of the culture ATPase activity was partially recovered. Delamere [38] showed the ability of lens epithelium to synthesize new Na,K-ATPase protein as a way to boost Na,K-ATPase in response to cell damage. Methionine incorporation studies indicated that Na,K-ATPase synthesis may also play a role in day to day preservation of high Na,K-ATPase activity.

Na,K-ATPase protein in lens epithelial cells appeared to be continually synthesized and degraded. This information may explain our results of ATPase recovery with time in culture after ending the exposure to heat of 39.5°C. In our study the recovery of ATPase activity 7 days after heating the lenses did not improve the structural damage to the lenses. The recovery of ATPase activity in culture conditions can be a result of the chaperone protection of alpha crystallin. Бlpha crystallin is a member of the small heat shock protein family. It is a molecular chaperone [39], which bind to proteins in the early stages of denaturation and sequesters them [40]. 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. [41] 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. [42] 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. In addition to its chaperon activity, alpha-crystallin is a major lens structural protein, which protects soluble enzymes against heat-induced aggregation and inactivation. Derham et al. [43] investigated the chaperone function of alpha-crystallin and its ability to protect the intrinsic membrane protein Na/K-ATPase in red blood cell ghosts from external stresses. They found that intracellular alpha-crystallin protected against inactivation induced by all external modifiers, in a dose-dependent manner. The lens is a closed system with a finite supply of alpha crystallin. Once the chaperone has been used in binding to other polypeptides, there is no more available. In human lenses above age 40

no free alpha crystallin can be detected [44]. There is a link between alpha crystallin content in the lens and lens flexibility as alpha crystallin decreases in the human lens, there is increase in stiffness of the lens nucleus as measured using dynamic mechanical analysis [45]. In vitro studies support this observation. Rao et al. [46] show that heating lens crystallins in the presence of alpha crystallin leads to the formation of high molecular weight proteins. When alpha crystallin is selectively removed the heated proteins became insoluble. Heat can be implicated in the damage that we see with age in human lenses. Heat can cause denaturation and unfolding of lens enzymes and lens structural proteins which increase lens stiffness and cataract formation.

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Резюме

ВОЗМОЖНОСТЬ УВЕЛИЧЕНИЯ РИСКА СТАРЕНИЯ ГЛАЗНОЙ ЛИНЗЫ ПРИ ВЛИЯНИИ ВЫСОКИХ ТЕМПЕРАТУР НА РАБОЧИХ МЕСТАХ, ТАКИХ КАК СТЕКОЛЬНЫЙ ЗАВОД, ПЕКАРНЯ.

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Цель: Катаракта (потеря прозрачности линзы) являются главной причиной слепоты в стареющем населении. Клинические наблюдения указали связь между высокой температурой и катарактой. Много рабочих подвержены высоким температурам в пекарнях и на стекольных фабриках. Наша цель состояла в том, чтобы исследовать механизмы, вовлеченные в повреждение высокой температурой на таких рабочих местах в линзе глаза.

Методы: Бычьи линзы были помещены в особенно разработанные контейнеры культуры для инкубации 2, 8, 11 и 14 дней. 160 линз использовались в исследовании, 20 линз для каждого опыта и 20 линз для контроля. Линзы были подвергнуты нагреванию при 39.5°C (пекар-

ня) и 37.8˚С (стекольный завод). Используя уникальный лазерный прибор, оптическое качество линз ежедневно оценивалось весь инкубационный период. В конце инкубации линзы были проанализированы и сфотографированы с помощью инвертет микроскопа, эпителиальный монослой был снят на предметное стекло использовался для гистохимического анализа АТФ-азной активности клеток.

Результаты: Интактные линзы подвергнутые ежедневно в условиях культуры нагреванию 37.8 ˚С на 75 мин. показывают увеличению в объеме эпителиальных клеток линзы и увеличение АТФ-азной активности. Хрусталики, подвергнутые 39.5 ˚С на 60 мин., 120 мин. в день в течение 2 дней, 120 мин. в день в течение 3 дневного воздействия показало повреждение эпителиальных клеток и уменьшение АТФ-азной активности.

Выводы: Наши результаты указывают, что вызванное высокой температурой повреждение хрусталика зависит от температуры и времени воздействия. Линзы, подвергнутые 37.8 ˚С, как на стекольном заводе, среагировали на напряжение повышением АТФ-азной деятельности. Хрусталики, подвергнутые 39.5 ˚С - уменьшением АТФ-азной активности. Ущерб был большим, когда время экспозиции было более длинным.

Ключевые слова: хрусталик, катаракта, здоровье работающих

Резюме

МОЖЛИВІСТЬ ЗБІЛЬШЕННЯ РИСКУ СТАРІННЯ ОЧНОЇ ЛІНЗИ ПРИ ВПЛИВІ ВИСОКИХ ТЕМПЕРАТУР НА РОБОЧИХ МІСЦЯХ, ТАКИХ ЯК СКЛЯНИЙ ЗАВОД, ПЕКАРНЯ

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Мета: Катаракта (втрата прозорості лінзи) є головною причиною сліпоти в старіючому населенні. Клінічні спостереження вказали зв’язок між високою температурою і катарактою. Багато робочих схильні до високих температур в пекарнях і на скляних фабриках. Наша мета полягала в тому, щоб досліджувати механізми, залучені в пошкодження високою температурою на таких робочих місцях в лінзі ока.

Методи: Бичачі лінзи були поміщені в особливо розроблені контейнери культури для інкубації 2, 8, 11 і 14 днів. 160 лінз використовувалися в дослідженні, 20 лінз для кожного досліду і 20 лінз для контролю. Лінзи були піддані нагріванню при 39.5 ˚С (пекарня) і 37.8 ˚С (скляний завод). Використовуючи унікальний лазерний прилад, оптична якість лінз щодня оцінювалася весь інкубаційний період. В кінці інкубації лінзи були проаналізовані і сфотографовані за допомогою инвертет-мікроскопа, епітеліальний моношар був знятий на предметне скло та використовувався для гістохімічного аналізу АТФ-азоної активності кліток.

Результати: Інтактні лінзи піддані щодня в умовах культури нагріванню 37.8 ˚С на 75 мин. показують збільшення в об’ємі епітеліальних кліток лінзи і збільшення АТФ-азної активності. Кришталики, піддані 39.5˚С на 60 мин., 120 мин. у день протягом 2 днів, 120 мин. в день протягом 3 денної дії показали пошкодження епітеліальних кліток і зменшення АТФ-азної активності.

Виводи: Наші результати указують, що викликане високою температурою пошкодження кришталика залежить від температури і часу дії. Лінзи, піддані 37.8 ˚С, як на скляному заводі, зреагували на напругу підвищенням АТФ-азної діяльності. Кришталики, піддані 39.5 ˚С - зменшенням АТФ-азної активності. Пошкодження зростало з ростом часу експозиції.

Ключові слова: хрусталік, катаракта, здоров’я працюючих

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