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Are Ultrasound-Guided Ophthalmic Blocks Injurious to the Eye? A Comparative Rabbit Model Study of Two Ultrasound Devices Evaluating Intraorbital Thermal and Structural Changes

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Abstract

Background—Since Atkinson's original description of retrobulbar block in 1936, needle-based anesthetic techniques have become integral to ophthalmic anesthesia. These techniques are unfortunately associated with rare, grave complications such as globe perforation. Ultrasound has gained widespread acceptance for peripheral nerve blockade but its translation to ocular anesthesia has been hampered because sonic energy, in the guise of thermal or biomechanical insult, is potentially injurious to vulnerable eye tissue. The United States Food and Drug Administration have defined guidelines for safe use of ultrasound for ophthalmic examination but most ultrasound devices used by anesthesiologists are not Food and Drug Administration-approved for ocular application because they generate excessive energy. Regulating agencies state that ultrasound examination can be safely undertaken as long as tissue temperatures do not increase $>1.5^{\circ}\text{C}$ above physiological levels.

Methods—Using a rabbit model, we investigated the thermal and mechanical ocular effects after prolonged ultrasonic exposure to single orbital and non-orbital-rated devices. In a dual-phase study, aimed at detecting ocular injury, the eyes of 8 rabbits were exposed to continuous 10-minute ultrasound examinations from two devices: 1) the Sonosite Micromaxx (non-orbital-rated) and 2) the Sonomed VuMax (orbital-rated) machines. In Phase I temperatures were continuously monitored via thermocouples implanted within specific eye structures ($n=4$). In Phase II the eyes were subjected to ultrasonic exposure without surgical intervention ($n=4$). All eyes underwent light microscopy examinations followed, at different intervals, by histology evaluations conducted by an ophthalmic pathologist.

Results—Temperature changes were monitored in the eyes of four rabbits. The non-orbital-rated transducer produced increases in ocular tissue temperature that surpassed the safe limit (increases $>1.5^{\circ}\text{C}$) in the lens of three rabbits (at 5.0, 5.5 and 1.5 minutes) and cornea of two rabbits (both at 1.5 minutes). A secondary analysis of temporal temperature differences between the orbital-rated

and non-orbital transducers revealed statistically significant differences (Bonferroni-adjusted $p < 0.05$) in the cornea at 3.5 minutes, the lens at 2.5 minutes and the vitreous at 4.0 minutes. Light microscopy and histology failed to elicit ocular injury in either group.

Conclusions—The non-orbital-rated ultrasound machine (Sonosite Micromaxx) increases the ocular tissue temperature. A larger study is needed to establish safety. Until then, ophthalmic blocks performed with ultrasound should be performed only with ocular-rated devices.

Introduction

Needle-based eye blocks depend on the periorbital injection of local anesthetic with needle trajectory guided by an anatomic knowledge of the orbit and contents. Atkinson first described retrobulbar injection of local anesthetics in 1936,¹ and the technique has remained popular despite rare catastrophic complications like globe puncture. The newer peribulbar block affords superior safety because needles remain outside the muscular cone.² Nonetheless, peribulbar block confers risk because needle tip position is not visualized.

Ultrasound has a well-established safety profile for ophthalmic examinations and is now a favored auxiliary for peripheral nerve blockade. The clinical introduction of ultrasound-guided ophthalmic anesthesia has been slow because no prospective studies address efficacy and safety. Because the eye is vulnerable to thermal and mechanical damage from excessive ultrasonic energy, the Food and Drug Administration (FDA) and World Federation for Ultrasound in Medicine and Biology have imposed strict thermal (TI) and mechanical index (MI) limits for ocular application (TI < 1.0 , MI < 0.23).^{3,4} Many operating room suite ultrasound devices used for peripheral nerve block are not orbital-rated. Given the current economic climate, it is unlikely facilities will purchase dedicated eye equipment.

In a rabbit model, we compared ocular physical and histopathology changes resulting from 10-minute exposures to an orbital-rated and a non-orbital-rated ultrasound device to determine whether a prolonged ultrasound exposure was associated with potential for sonic-induced ocular injury (thermal or mechanical).

Materials and Methods

The study received approval from the University of Miami Institutional Animal Care and Use Committee (IACUC Protocol 09-137). This was a dual phase, comparative rabbit model study addressing the potential for ultrasound-induced orbital thermal and structural injury after prolonged exposure. In ophthalmic research, the rabbit has been an accepted translational model for the human eye.⁵ While our literature search did not find an animal study that measures ultrasound-induced ocular thermal changes, we discovered one study based upon theoretical criteria.⁶ Accordingly, we compared the thermal and mechanical changes produced by an ophthalmic-rated (orbital-rated) apparatus (Sonomed VuMaxx) [Lake Success, NY, USA], and a nonrated (non-orbital-rated) portable device (Sonosite Micromaxx) [Bothell, WA, USA]. The latter was chosen because, at the time of this study, regional anesthesiologists commonly used this device for peripheral nerve blockade.

During Phase I, four rabbits were anesthetized using a standard mixture of IV ketamine 35 mg/kg, xylazine 5 mg/kg and acepromazine 0.75 mg/kg. An initial light microscopy examination, to exclude preexisting pathology, was performed by an ophthalmologist. Four Type “T” 0.005 inch (copper-constantan) diameter Teflon coated thermocouples (Omega, Stamford, CT) were surgically implanted at intracorneal, intracameral, intravitreal and subcutaneous sites for continuous thermal data acquisition. An active ultrasound transducer was manually applied to the nonlubricated cornea for ten minutes. The operator attempted to maintain the transducer in a stationary position. In each animal, the left eye (OS) was

exposed to sound waves generated by the orbital-rated ultra-high frequency (40MHz) transducer (MI and TI <0.3), and the right eye (OD) exposed to the non-orbital-rated P10 (8-4MHz) probe (MI = 1.0 & TI = 0.8). After ultrasound exposure all eyes were re-examined under light microscopy. The animals were then killed, and their eyes submitted for histopathologic evaluation.

Phase II involved no surgical interventions. In an analogous manner, four rabbits were anesthetized using the same cocktail. An ophthalmologist performed preinvestigation light microscopy to exclude preexisting conditions, and then each eye was exposed to ten minutes of ultrasound from either the orbital-rated or non-orbital-rated device. After exposure, the eyes were re-examined under light microscopy. In the next 72 hours these animals underwent daily light microscopy examinations. At postoperative day 3 the animals were killed and eyes were submitted for final histopathologic evaluation.

A control group consisted of two anesthetized rabbits. In each animal one eye underwent 10-minute corneal application of an inactive transducer followed by light microscopy and ocular histopathology examination. Our rationale was to exclude injury from physical application of the transducer. Tissue insult was categorized according to a four-grade scale [normal (0), edema (+), inflammatory (++), and hemorrhage (+++)].

Statistical Methods

The corneal, cameral, vitreous, and subcutaneous temperatures were continuously measured in each eye over a ten-minute period in four rabbits. These data were averaged over 30 second intervals for analyses. Our primary analysis was to detect increases in tissue temperatures of >1.5° C. For this analysis, we examined the data of each of the four rabbits separately to determine the time at which the temperature exceeded the baseline temperature by 1.5° C in each of the four areas in each eye. The results were reported as mean time in minutes with a lower 95% confidence interval.

A secondary analysis compared the OD, OS, and subcutaneous temperatures in the cornea, lens and vitreous. A linear mixed model was used to analyze a within-animal design that included area (OD, OS, or subcutaneous), minute (0.5 to 10.0 by 0.5 increments), and the area by minute interaction as the within-animal effects. The rabbits were considered random, and area and minute were fixed. An autoregressive single lag covariance matrix was used to accommodate the correlated covariance structure. Contrasts were used to compare the OD and OS temperatures with the subcutaneous temperature at each interval and OD and OS temperatures at each time. The p-values of the significance tests for each set of contrasts between 2 groups for each of the 20 time measures were adjusted for multiple comparisons using the Bonferroni technique, yielding a two-tailed alpha of 0.05/(2 × 20). Least squares means, standard errors, and p-values are presented in the tables. SAS 9.2 (SAS Institute, Inc., Cary, NC) was used for all analyses. The 0.05 level was used to determine significance.

Results

Phase I (Thermal)

In four rabbits, temperature changes during the 10-minute ultrasound exposure were continuously monitored at the three implanted intraocular locations (cornea, lens and vitreous), and subcutaneously. Graphs generated for the orbital-rated and non-orbital-rated devices are depicted in Figures 1 and 2. In the non-orbital-rated subjects, the rate of temperature increase was greater and more closely approximated subcutaneous temperatures.

A characteristic of the orbital-rated was an initial phase of tissue cooling followed by modest sustained temperature increases (Figure 1). The largest thermal increases were in the vitreous compartment but all chambers slowly approximated the subcutaneous temperature. In the rabbits monitored for temperature changes, the non-orbital-rated probe produced immediate temperature increases in all three compartments with earlier equilibration towards the subcutaneous temperature (Figure 2). Importantly, in these four animals, the 1.5° C limit for increase in tissue temperature was reached in the lens of three animals (at 5.0, 5.5 and 1.5 minutes, respectively), and the cornea of two at 1.5 minutes (for both rabbits).

Light microscopy examinations of eyes exposed to both ultrasonic transducers were normal, except for animal R09-255 OD in which corneal edema occurred secondary to erroneous application of Aquasonic® transmission gel.

An ophthalmic pathologist, unaware of prior intervention, reviewed the histology. The process of implanting thermocouples is intrusive, and may have accounted for hemorrhagic and traumatic injury witnessed in three phase one animals (R09-255, R09-256, and R09-257). In animals without thermocouple implantation there was no evidence of tissue damage.

Statistical Analysis (Thermal)

In all compartments, recorded temperatures remained lower than subcutaneous temperatures. The compartments of those eyes exposed to the non-orbital-rated transducer became warmer than those exposed to the orbital-rated transducer. The temperature differences became significant at 3.5 minutes for cornea, 2.5 minutes for lens and 4.0 minutes for vitreous (Tables 1, 2, 3).

Phase II (Mechanical)

In phase II evidence was sought, in four rabbits (R09 259-262), for ultrasound-induced tissue injury over a 72-hour period. All examinations were normal, except for corneal edema in animal R09-260. An ophthalmic pathologist prepared and examined the histology slides in an analogous manner to phase I. There were no histological changes in either the orbital-rated or non-orbital-rated groups (Figures 3,4,5).

Discussion

We tested the hypothesis that the ocular thermal and mechanical impacts of exposure to one popular operating room non-orbital-rated ultrasound device are similar to an ophthalmic-rated biometry machine. We aimed to establish whether energy emissions from this device induce ocular injury. We used the rabbit, an accepted translational model for the human eye.^{5,7}

In 1995 Birch et al used ultrasound to elicit the proximity of a needle tip to the back of the eye during retrobulbar block.⁸ Eye blocks were performed by ophthalmologists who assessed posterior globe to needle tip distance at > 5 mm. Subsequent ultrasound examinations revealed needles were significantly closer to the globe (some as near as 0.2mm). Furthermore, the needle shaft had indented the eye in many cases.

The eye is a sensory organ that is vulnerable to sonar damage.⁹ Accordingly, the FDA has set strict guidelines for ocular ultrasonic application. Before passage of the Medical Device Act of 1976, the maximum permissible acoustic energy intensity for ocular applications was 17mW/cm² (spatial-peak temporal average).³ With adoption of the Output Display Standard this level was increased to 50mW/cm².^{6,10} Acoustic power output is the primary

determinant of the TI and MI. These indices approximate risk for adverse biological effects with values < 1 considered safe. Newer devices (such as the Sonosite M-Turbo) feature an ocular setting and the ability to decrease power output ($MI=0.2$, $TI=0$). Currently, the FDA sets no time constraint for performing ocular examinations.³

Thermal Index (TI)

The TI is the ratio of the total device acoustic power to the power required to increase tissue temperature by 1°C (Figure 6). It may be further subcategorized into tissue type viz. soft tissue, bone and cranial bone.¹¹ Multiple factors including beam and scanning variables affect heat generation.¹² According to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB), a diagnostic procedure can be safely undertaken if tissue temperature increases $< 1.5^{\circ}\text{C}$ above physiological levels.¹³

Biological structures are exposed to physiological shifts of 3°C without permanent injury.¹⁴ Hatab et al found that if ultrasound increased tissue temperature by 4°C , then injury occurred after 16-minute exposures. However, when temperature increased by 6°C , then changes occurred at one minute.¹⁵ Orbital-rated ultrasound bio-microscopy transducers are different from standard transducers because they generate high frequency sound waves (35-50MHz), and use a water bath that cools the surface.¹⁶

The non-orbital-rated transducer produced temperature increases in corneal and cameral tissues of $>1.5^{\circ}\text{C}$ (the EFSUMB limit) beyond 8 minutes. However, there were significant tissue temperature differences across the transducer types after 2.0 minutes ($p < 0.001$) (Tables 1,2,3).

Mechanical Index (MI)

The MI estimates potential for macro streaming and cavitations, a process in which ultrasonic vibrations produce tiny gas bubbles¹⁷ (Figure 7). These biophysical phenomena have been studied in vitro¹³ but the in vivo relationship between acoustic output and biological effect is unclear. Furthermore, these effects are undocumented in humans.^{6,12}

In this study both transducer types caused increases in intraocular temperature that surpassed EFSUMB safety limits (Figures 1 and 2). Nonetheless, we failed to elicit macro- or microscopic evidence of tissue damage (Figures 3 and 4). Our outcomes are in agreement with those of Silverman et al and Cucevic et al who demonstrated wide safety margins for high frequency orbital ultrasound examinations.^{14,18}

Histological aberrations were only evidenced in eyes that underwent thermocouple implantation (Figure 5). The nonsurgical specimens demonstrated no microscopic injury (Figures 3 and 4).

Our study has several methodological limitations. We applied the transducer directly on the cornea because, unlike humans, the rabbit eyelid retracts. This promoted heat transfer and potential for contact injury. Also, we used an older model ultrasound device that lacked capability to reduce its power output. Finally, detection of cellular injury may have been difficult given the small population, low incidence of ultrasonic-induced harm, use of a single model transducer, and absence of long-term and electron-microscopy assessments. This study cannot establish the true limits of safety due to the small number of animals studied.

Conclusions

In a rabbit model, we report that the non-ocular-rated ultrasound machine increased the ocular tissue temperature. Until a larger study establishes safety, non-ocular-rated devices should not be used for ultrasound-guided ophthalmic blocks. The data suggest that after safety has been established, a device may be suitable for brief periods (e.g., < 90 sec). For now, ultrasound-guided ophthalmic blocks should only be performed with ocular-rated devices.

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References

1. Atkinson WS. Retrobulbar injection of anesthetic within the muscular cone. *Arch Ophthalmol.* 1936; 16:494–503.
2. Davis DB 2nd, Mandel MR. Efficacy and complication rate of 16,224 consecutive peribulbar blocks: A prospective multicenter study. *J Cataract Refract Surg.* 1994; 20:327–37. [PubMed: 8064611]
3. Food and Drug Administration. Guidance for Industry and FDA Staff Information for Manufacturers Seeking Marketing Clearance of Diagnostic Ultrasound Systems and Transducers. Silver Springs; Maryland: Sep 9. 2008
4. Barnett SB. World Federation for Ultrasound in Medicine and Biology (WFUMB) Symposium on safety of ultrasound in medicine: Conclusions and recommendations on thermal and non-thermal mechanisms for biological effects of ultrasound. *Ultrasound Med Biol.* 1998; 24:1–55. [PubMed: 9483765]
5. Berry M, Easty DL. Isolated human and rabbit eye: Models of corneal toxicity. *Toxicol in Vitro.* 1993; 7:461–4.
6. Herman BA, Harris GR. Theoretical Study of steady-state temperature rise within the eye due to ultrasound insonation. *IEEE Trans Ultrason Ferroelectr Freq Control.* 1999; 46:1566–74. [PubMed: 18244355]
7. Mencucci R, Ambrosini S, Ponchietto C, Marini M, Vannelli GB, Menchini U. Ultrasound thermal damage to rabbit corneas after simulated phacoemulsification. *J Cataract Refract Surg.* 2005; 31:2180–6. [PubMed: 16412936]
8. Birch AA, Evans M, Redembo E. The ultrasonic localization of retrobulbar needles during retrobulbar block. *Ophthalmol.* 1995; 102:824–6.
9. Barnett SB, Rott HD, ter Haar GR, Ziskin MC, Maeda K. The sensitivity of biological tissue to ultrasound. *Ultrasound Med Biol.* 1997; 23:805–12. [PubMed: 9300983]
10. American Institute of Ultrasound in Medicine. How to interpret the ultrasound output display standard for higher acoustic output diagnostic ultrasound devices: version 2. *J Ultrasound Med.* 2004; 23:723–6. [PubMed: 15154543]
11. Leaming DV. Practice styles and preference of ASCRS members. *J Cataract Refract Surg.* 2004; 30:892–900. [PubMed: 15093657]
12. Dalecki D, Raeman CH, Child SZ, Carstensen EL. Intestinal hemorrhage from exposure to pulsed ultrasound. *Ultrasound Med Biol.* 1995; 21:1067–72. [PubMed: 8553501]
13. European Federation of Societies for Ultrasound in Medicine and Biology. European Committee For Medical Ultrasound Safety Bylaw. Newsletter. 1998; 12
14. Cucevic C, Brown AS, Foster F Stuart. Thermal Assessment of 40-MHz Pulsed Doppler Ultrasound in Human Eye. *Ultrasound Med Biol.* 2005; 31:565–73. [PubMed: 15831335]
15. Hatab MR, Giller CA, Clarke GD. Evaluation of cerebral arterial flow with transcranial doppler ultrasound: Theoretical development and phantom studies. *Ultrasound Med Biol.* 1997; 23:1025–31. [PubMed: 9330446]

16. Carstensen EL, Child SZ, Norton S, Nyborg WL. Ultrasonic heating of the skull. *J. Acoust Soc Am.* 1990; 87:1310–17. [PubMed: 2182690]
17. Duck FA. Hazards, risks and safety of diagnostic ultrasound. *Medical Engineering and Physics.* 2008; 30:1338–48. [PubMed: 18635388]
18. Silverman RH, Lizzi FL, Ursea BG, Cozzarelli L, Ketterling JA, Deng CX, Folberg R, Coleman DJ. Safety levels for exposure of cornea and lens to very high frequency ultrasound. *J Ultrasound Med.* 2001; 20:979–86. [PubMed: 11549159]

Figure 1.
Ultrasound bio-microscopy: Ocular compartmental actual temperature ($^{\circ}\text{C}$) vs. time (mins)

Figure 2.
Sonosite: Ocular compartmental actual temperature ($^{\circ}\text{C}$) vs. time (mins)

Figure 3.
Ultrasound bio-microscopy: Histopathology: (1) Cornea (2) Cameral (3) Retina

Figure 4.
Sonosite: Histopathology: (1) Cornea (2) Cameral (3) Retina

Histopathology Evaluation

Figure 5.
Histopathology: Composite analysis 72 hours after exposure

$$TI = W_p / W_{deg},$$

W_p = power (mW)

W_{deg} = estimated power (mW) necessary to raise target tissue 1°C.

Figure 6.
Thermal Index Equation

$$MI = \frac{P_{r.3'} (fawf^{1/2})}{C_{MI}}$$

$P_{r.3}$ = attenuated peak-rarefactional pressure

$fawf$ = acoustic working frequency

C_{MI} = normalizing coefficient: $1\text{MPa MHz}^{-1/2}$

Figure 7.
Mechanical Index Equation

Table 1

Comparative cornea temperature vs. time (min).

Time	Subcutaneous	OD	OS	SQ vs. OD	SQ vs. OS	OD vs. OS
0.5	35.58 ± 1.09	31.08 ± 1.09	31.58 ± 1.09	0.050	0.039	1.000
1.0	35.68 ± 1.09	31.30 ± 1.09	31.34 ± 1.09	0.064	0.016	1.000
1.5	35.65 ± 1.09	31.79 ± 1.09	31.14 ± 1.09	0.184	0.010	1.000
2.0	35.64 ± 1.09	31.99 ± 1.09	30.13 ± 1.09	0.277	0.001	1.000
2.5	35.60 ± 1.09	31.62 ± 1.09	28.76 ± 1.09	0.143	<0.001	0.514
3.0	35.60 ± 1.09	31.51 ± 1.09	27.89 ± 1.09	0.117	<0.001	0.098
3.5	35.62 ± 1.09	32.02 ± 1.09	27.83 ± 1.09	0.300	<0.001	0.024
4.0	35.63 ± 1.09	32.20 ± 1.09	27.49 ± 1.09	0.399	<0.001	0.006
4.5	35.62 ± 1.09	32.20 ± 1.09	27.11 ± 1.09	0.410	<0.001	0.002
5.0	35.60 ± 1.09	32.23 ± 1.09	27.24 ± 1.09	0.442	<0.001	0.003
5.5	35.60 ± 1.09	32.09 ± 1.09	27.52 ± 1.09	0.342	<0.001	0.009
6.0	35.61 ± 1.09	32.01 ± 1.09	27.62 ± 1.09	0.300	<0.001	0.014
6.5	35.60 ± 1.09	32.06 ± 1.09	27.45 ± 1.09	0.325	<0.001	0.008
7.0	35.59 ± 1.09	32.02 ± 1.09	27.30 ± 1.09	0.316	<0.001	0.006
7.5	35.56 ± 1.09	32.25 ± 1.09	27.60 ± 1.09	0.489	<0.001	0.007
8.0	35.56 ± 1.09	32.91 ± 1.09	28.28 ± 1.09	1.000	<0.001	0.007
8.5	35.57 ± 1.09	33.46 ± 1.09	28.51 ± 1.09	1.000	<0.001	0.003
9.0	35.58 ± 1.09	33.59 ± 1.09	28.76 ± 1.09	1.000	<0.001	0.004
9.5	35.57 ± 1.09	33.51 ± 1.09	29.47 ± 1.09	1.000	<0.001	0.036
10.0	35.55 ± 1.09	33.25 ± 1.09	29.31 ± 1.09	1.000	<0.001	0.044

Least squares mean ± standard error

Each set of 20 comparison p-values are Bonferroni corrected

From 8-10 minutes the OD temperature is more than 1.5 degrees higher than at 0.5 minutes

OS = left eye

OD = right eye

Table 2

Comparative cameral temperature vs. time (min).

Time	Subcutaneous	OD	OS	SQ vs. OD	SQ vs. OS	OD vs. OS
0.5	35.58 ± 0.88	32.29 ± 0.88	31.51 ± 0.88	0.143	0.005	1.000
1.0	35.68 ± 0.88	32.26 ± 0.88	31.33 ± 0.88	0.107	0.002	1.000
1.5	35.65 ± 0.88	32.40 ± 0.88	30.68 ± 0.88	0.160	<0.001	1.000
2.0	35.64 ± 0.88	32.55 ± 0.88	29.27 ± 0.88	0.236	<0.001	0.054
2.5	35.60 ± 0.88	32.33 ± 0.88	28.16 ± 0.88	0.151	<0.001	0.003
3.0	35.60 ± 0.88	32.45 ± 0.88	27.14 ± 0.88	0.205	<0.001	<0.001
3.5	35.62 ± 0.88	32.64 ± 0.88	26.83 ± 0.88	0.300	<0.001	<0.001
4.0	35.63 ± 0.88	32.83 ± 0.88	26.87 ± 0.88	0.431	<0.001	<0.001
4.5	35.62 ± 0.88	32.91 ± 0.88	26.84 ± 0.88	0.514	<0.001	<0.001
5.0	35.60 ± 0.88	32.98 ± 0.88	26.77 ± 0.88	0.626	<0.001	<0.001
5.5	35.60 ± 0.88	33.14 ± 0.88	26.65 ± 0.88	0.853	<0.001	<0.001
6.0	35.61 ± 0.88	33.28 ± 0.88	26.96 ± 0.88	1.000	<0.001	<0.001
6.5	35.60 ± 0.88	33.28 ± 0.88	27.67 ± 0.88	1.000	<0.001	<0.001
7.0	35.59 ± 0.88	33.28 ± 0.88	28.23 ± 0.88	1.000	<0.001	<0.001
7.5	35.56 ± 0.88	33.55 ± 0.88	28.72 ± 0.88	1.000	<0.001	<0.001
8.0	35.56 ± 0.88	34.05 ± 0.88	29.29 ± 0.88	1.000	<0.001	<0.001
8.5	35.57 ± 0.88	34.41 ± 0.88	29.67 ± 0.88	1.000	<0.001	<0.001
9.0	35.58 ± 0.88	34.58 ± 0.88	29.99 ± 0.88	1.000	<0.001	0.001
9.5	35.57 ± 0.88	34.28 ± 0.88	30.19 ± 0.88	1.000	<0.001	0.005
10.0	35.55 ± 0.88	33.86 ± 0.88	30.30 ± 0.88	1.000	<0.001	0.024

Least squares mean ± standard error

Each set of 20 comparison p-values are Bonferroni corrected

From 8-10 minutes the OD temperature is more than 1.5 degrees higher than at 0.5 minutes

OS = left eye

OD = right eye

Table 3

Comparative vitreous temperature vs. time (min).

Time	Subcutaneous	OD	OS	SQ vs. OD	SQ vs. OS	OD vs. OS
0.5	35.58 ± 0.72	34.88 ± 0.72	34.25 ± 0.72	1.000	1.000	1.000
1.0	35.68 ± 0.72	34.89 ± 0.72	34.30 ± 0.72	1.000	0.915	1.000
1.5	35.65 ± 0.72	34.93 ± 0.72	34.23 ± 0.72	1.000	0.834	1.000
2.0	35.64 ± 0.72	34.98 ± 0.72	33.74 ± 0.72	1.000	0.139	1.000
2.5	35.60 ± 0.72	34.82 ± 0.72	33.16 ± 0.72	1.000	0.012	0.360
3.0	35.60 ± 0.72	34.70 ± 0.72	32.70 ± 0.72	1.000	0.001	0.090
3.5	35.62 ± 0.72	34.87 ± 0.72	32.75 ± 0.72	1.000	0.001	0.054
4.0	35.63 ± 0.72	34.98 ± 0.72	32.76 ± 0.72	1.000	0.001	0.033
4.5	35.62 ± 0.72	35.01 ± 0.72	32.66 ± 0.72	1.000	0.001	0.018
5.0	35.60 ± 0.72	35.01 ± 0.72	32.39 ± 0.72	1.000	<0.001	0.005
5.5	35.60 ± 0.72	35.03 ± 0.72	32.18 ± 0.72	1.000	<0.001	0.001
6.0	35.61 ± 0.72	35.03 ± 0.72	32.21 ± 0.72	1.000	<0.001	0.002
6.5	35.60 ± 0.72	35.07 ± 0.72	32.47 ± 0.72	1.000	<0.001	0.005
7.0	35.59 ± 0.72	35.14 ± 0.72	32.66 ± 0.72	1.000	0.001	0.009
7.5	35.56 ± 0.72	35.19 ± 0.72	32.73 ± 0.72	1.000	0.001	0.011
8.0	35.56 ± 0.72	35.23 ± 0.72	32.81 ± 0.72	1.000	0.002	0.013
8.5	35.57 ± 0.72	35.26 ± 0.72	32.89 ± 0.72	1.000	0.003	0.017
9.0	35.58 ± 0.72	35.30 ± 0.72	32.95 ± 0.72	1.000	0.005	0.018
9.5	35.57 ± 0.72	35.32 ± 0.72	32.92 ± 0.72	1.000	0.004	0.015
10.0	35.55 ± 0.72	35.29 ± 0.72	32.91 ± 0.72	1.000	0.004	0.016

Least squares mean ± standard error

Each set of 20 comparison p-values are Bonferroni corrected.

OS = left eye

OD = right eye