Intraocular pressure in the smallest primate aging model: the gray mouse lemur

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Intraocular pressure in the smallest primate aging model, the gray mouse lemur

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Keywords:	intraocular pressure, tonometer, reference value, mouse lemur, primate, aging

Figure 1. Setup of the manometrical investigation.
1151x863mm (72 x 72 DPI)

Figure 2. Investigation of a 2 year old mouse lemur with the TonoVet®.
283x179mm (300 x 300 DPI)

Figure 3. Investigation of a 2 year old mouse lemur with the TonoPen™
1024x685mm (96 x 96 DPI)

Review

Figure 4. Values measured with the TonoVet® and TonoPen™ under manometrical control. Manometrically determined IOP for the eight eyes of four animals are represented on the x-axis. TonoVet® and TonoPen™ values are represented on the y-axis. Each dot represents the mean from three measurements per step/per eye. The dashed line represents the regression line for the TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) and TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) measurements.

73x64mm (300 x 300 DPI)

Figure 5. Comparison of the in-vivo measured IOP for the TonoVet® and TonoPen™ in 12 animals. The results show high variation in measurements for the TonoPen™ and much more consistent values for the TonoVet®. E.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). For exact values (see Tab. 1).
91x99mm (300 x 300 DPI)

Figure 6. Relation between tIOP values and age in healthy mouse lemur eyes. This scatterplot shows the tIOP values for all measured healthy animals from Hannover and Montpellier (N = 258) on the y-axis in relation to age on the x-axis. The decrease in IOP is statistically not significant ($p = 0.077$, $r_s = -0.110$).
74x65mm (300 x 300 DPI)

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Intraocular pressure in the smallest primate aging model, the gray mouse lemur

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3 Suggested running title: Intraocular pressure in aging mouse lemurs

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Abstract

Objective: The aim of this study was to assess the practicability of common tonometers used in veterinary medicine for rapid IOP screening, to calibrate IOP values gained by the tonometers and to define a reference IOP value for the healthy eye in a new primate model for aging research, the gray mouse lemur.

Studied animals & Procedures: TonoVet® and the TonoPen™ measurements were calibrated manometrically in healthy enucleated eyes of mouse lemurs euthanized for veterinary reasons. For comparison of the practicability of both tonometers as a rapid IOP assessment tool for living mouse lemurs, the IOP of 24 eyes of 12 hand-fixed animals (six males and six females) was measured. To define a standard reference value for IOP in healthy mouse lemurs, two of the largest colonies in the world were examined using the most practicable tonometer.

Results: IOP measurements for the TonoVet® can be corrected by the formula: $y = 0.981 + (1.962 * \text{TonoVet}^{\circledR} \text{ value})$, for the TonoPen™ by $y = 5.38 + (1.426 * \text{TonoPen}^{\text{TM}} \text{ value})$. The calibrated IOP for a healthy mouse lemur eye is 20.3 ± 2.85 mmHg. The TonoVet® showed advantages in practicability, e.g. small corneal contact area, short and painless corneal contact and time. IOP measurements of healthy mouse lemur eyes were not affected by age, sex, eye side or colony.

Conclusion: Tonometry using TonoVet® is the more practicable assessment tool for IOP measurement of the tiny eyes of living mouse lemurs. Pathological deviations can be identified based on the described reference value.

Key words: intraocular pressure, tonometer, reference value, mouse lemur, primate, aging

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51 **Introduction**

52 Mouse lemurs belong to the smallest living primates worldwide. (1) Due to this fact, their
53 maintenance and breeding is more cost-efficient than in larger primate species and they are
54 also not known for spreading zoonotic diseases which makes them an extraordinary primate
55 model for research. Additionally mouse lemurs have a life expectancy which is much shorter
56 than in other non-human primate aging models with about 8 years in the wild and up to 18.5
57 years in captivity. (2, 3) The genome of mouse lemurs has recently also been sequenced by
58 the Broad Institute (GenBank accession number ABDC000000000). Besides of the importance
59 of mouse lemurs for biomedical as well as aging research, (4-9) they are also important for
60 evolutionary research, based on their high and cryptic species diversity, their uneven
61 distribution and their flexible adaptations to their natural habitats. (10)
62 Mouse lemurs are nocturnal and have relative small absolute eye sizes with 9.4 mm in
63 diameter. (11, 12) Aged gray mouse lemurs were reported to suffer from different eye
64 diseases such as cataract, retinal atrophy and buphthalmia, an abnormal enlargement of the
65 eyeball. (13) Whether this malformation was due to glaucoma, which causes ocular
66 hypertension, still need to be clarified though. Because of the difficulty in handling non-
67 anesthetized animals, however, IOP was never studied in mouse lemurs before. Applanation
68 and rebound tonometry are commonly used in veterinary medicine to determine IOP in
69 domestic animals such as dogs, (14, 15) cats, (16-18) and birds (19, 20) as well as in
70 laboratory animals such as rats, (21, 22) rabbits (23) and macaques. (24, 25) Since factory
71 settings for TonoPen™ and TonoVet® are only available for common species in the
72 veterinary clinics such as dogs, cats and horses, for uncommon species it is necessary to
73 calibrate measurements by manometry to get a true IOP (tIOP) before defining standard
74 reference IOP values for a given species. (see e.g. rabbits, (26) birds (20) and macaques. (25))

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3 75 The TonoPen™ is an applanation tonometer often used for intraocular measurements in
4
5 76 veterinary medicine. (14, 16, 21) IOP measurement gives an indirect assessment of the IOP
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7 77 by using the Imbert-Fick law. (27) It measures the counter pressure that is necessary to flatten
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9 78 a thin membrane surrounding a sphere filled with liquid. Its use in dogs and cats is easy and
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11 79 fast but requires a local anaesthesia of the cornea.

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14 80 The TonoVet® is a rebound tonometer based on a patented measurement system which uses
15
16 81 a small, disposable probe which is brought into contact with the cornea. (17, 18, 20, 24, 28)
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18 82 The probe is rebounding with a determined speed, correlating to the IOP. The higher the IOP
19
20 83 is, the higher the speed of the return-bounce. Its use is easy and fast and requires no local
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22 84 anaesthesia.

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25 85 In this study we applied TonoPen™ and TonoVet® as rapid IOP assessment tools to the gray
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27 86 mouse lemur, to

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30 87 1. calibrate IOP measurements of the tonometers by manometry,
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32 88 2. assess the practicability of the tonometers to measure the IOP of mouse lemurs' eyes
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34 89 in-vivo to screen colonies,
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36 90 3. apply the most practicable technique for screening IOP in two of the world's largest
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38 91 colonies, to investigate the effect of eye position, sex, colony and age on IOP and
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40 92 establish a reference value for IOP.

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100 **Methods**

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102 **Animals and maintenance**

103 Mouse lemurs (*Microcebus murinus*) tested in this study belonged to two licensed breeding
104 colonies housed at the Institute of Zoology at the University of Veterinary Medicine
105 Hannover (for details in housing conditions see; (29) Hannover breeding licence number
106 42500/1H) and the University of Montpellier 2 (Agreement N^{br} ≠ C-34-172-23). Out of 349
107 investigated animals 258 animals, which showed no ocular pathologies, have been used for
108 analysis, 75 (38 females; 37 males) from Hannover and 183 (101 females; 82 males) from
109 Montpellier, ranging from 0.5 to 10 years. All animals were born in captivity. Since mouse
110 lemurs are nocturnal, the captive animals were maintained under artificial light conditions
111 with a reversed light cycle. Additionally, animals in Montpellier are maintained under an
112 accelerated photoperiodic regime. This means that the photoperiodically triggered
113 reproductive “year” lasted 8 instead of 12 months. It has been shown that these conditions
114 accelerate aging processes in gray mouse lemurs by the factor 1.5. (30-32)

115

116 **Ophthalmologic investigation**

117 Handling for ophthalmological examinations was similar to the weekly caretaker handling of
118 the animals resulting in reduced stress for the lemurs. All examinations were conducted at the
119 end of the sleeping period/beginning of the activity period to minimize disturbances of the
120 animal’s activity. All procedures applied in this study were licenced by the respective
121 authorities (Hannover licence number, 33.9-42502-05-11A200, LAVES to Elke
122 Zimmermann; Montpellier licence number, 34-124 to Jean-Michel Verdier).

123

Both eyes of a lemur were investigated with a slit-lamp bio-microscope (SL-14; Kowa, Eickemeyer, Germany) and indirect ophthalmoscope (Omega 100; Heine, Ettenheim, Germany) to determine potential eye pathologies with a possible effect on IOP or corneal consistence. To get a view on the lens and retina, mydriatic eye-drops (Mydrum®, Chauvin ankerpharm GmbH, Berlin, Germany) were used to widen the pupil.

1. Manometry

We determined the IOP value of an enucleated eye with the TonoVet® and TonoPen®, respectively, at manometrically defined IOP pressure steps (DD-890, ATP Messtechnik GmbH, Ettenheim, Germany). The used manometer was calibrated by the bureau of standards in Hannover (Mess- und Eichwesen Niedersachsen Betriebsstelle Eichamt Hannover, Goethestraße 44, 30169 Hannover). The pressure measured by the manometer in this setup (including the pressure in the examined eye) will be labelled as the true IOP (tIOP). The values in the more relevant sector for clinical use between 5 mmHg and 50 mmHg have been taken in steps of 5 mmHg $\pm$ 0.1 mmHg. Between 50 mmHg and 100 mmHg measurements were taken in steps of 10 mmHg $\pm$ 0.1 mmHg.

Eight healthy eyes of four animals euthanized for veterinary reasons (incurable pathologies) were enucleated transconjunctivally immediately after euthanasia. These eyes were called healthy since they were found inconspicuous and showed no signs of pathological disease according to an ophthalmological investigation performed not more than 6 months before.

After enucleation, the eyes were stored in 0.9% NaCl solution at 6°C for up to a maximum of 4 hours before measurement. A small bowl of dough was adjusted to ensure the fixation of the enucleated eye. The cannula (24 G, length 25 mm, B. Braun Melsungen AG, D-34209) was inserted transsclerally into the vitreous and was not moved or reinserted while taking the measurement. The pressure was constant and measured values showed no fluctuation.

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149 Minimal leakages were observed sporadically, but sealed by themselves with higher pressure.
150 A three-way stopcock was connected with the cannula, the manometer and a NaCL solution
151 reservoir via three silicon tubes. Pressure was adjusted by changing the height of the NaCl
152 solution reservoir. The whole system was opened all the time to avoid fluctuations. (see Fig.
153 1) Once the manometer displayed a constant pressure, the pressure was measured using the
154 TonoVet® and TonoPen™. Each complete measurement by the TonoVet® represented the
155 mean of four single values, for the TonoPen™ the mean consists out of four to five single
156 values. The measurement was carried out until three complete measurements per eye were
157 successfully obtained.

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160 **2. In-vivo application of TonoPen™ and TonoVet®**

161 The tonometers TonoPen™ and TonoVet® were used to measure IOP in the eyes of 12
162 young mouse lemurs (6 males, 6 females; aged between 2 and 3 years; Colony Hannover).
163 Both eyes of these mouse lemurs were inconspicuous and showed no signs of pathological
164 diseases according to an ophthalmological investigation performed one day before.
165 Measurements of the TonoVet® and TonoPen™ have been taken 24 hours after the
166 ophthalmological investigation to minimize influences of the mydriatic eye-drops on the IOP.
167 Each animal was investigated on both eyes until a successful measurement with the
168 respective tonometer has been achieved. The successful measurement is indicated by an
169 acoustical signal.

2.a Rebound tonometry (TonoVet®)

Six single values of the IOP were taken per eye by the TonoVet® tonometer (TonoVet®; ICare, Finland Oy). The TonoVet® then automatically deleted the lowest and highest value and calculated the mean out of the remaining four values. Only successfully completed measurements displaying a mean on the TonoVet® were recorded. One successfully completed measurement per animal was taken. For all 12 animals no anaesthesia and no forced fixation of the eyelids were necessary (see Fig. 2).

2.b Applanation tonometry (TonoPen™)

The TonoPen™ tonometer (TonoPen™; Reichert® Technologies, Eickemeyer, Germany) was used to measure IOP of the same 12 animals as for the TonoVet®. Before measurements were taken, the eyes were locally anaesthetized with eye-drops (Proparacain-POS® 0.5%). To prevent a reflective wink of the eyelids of an animal, the examiner had to fix the eyelids with his fingers in open position (see Fig. 3). Between four and five single values per eye were necessary for the TonoPen™ to calculate a mean. Only successfully completed measurements displaying a mean on the TonoPen™ were recorded. One successfully completed measurement per animal was taken.

3. Determination of IOP in two colonies

To determine a reference value for the healthy mouse lemur eye, a large sample size is required. 241 animals in Montpellier and 108 animals in Hannover have been investigated. For the determination of the IOP the TonoVet® was used. The animals were investigated at the end of the sleeping/beginning of the activity period. For Montpellier the time for investigation ranged from 09:00 a.m. – 03:00 p.m. (beginning of activity period at 12:00 a.m. for all

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199 animals), for Hannover from 09:00 a.m. – 05:00 p.m. (beginning of activity period at 10:00
200 a.m., 12:00 a.m. or 02:00 p.m. respectively, according to the room). After IOP measurement,
201 an ophthalmological investigation was performed for each animal as described in the
202 paragraph ophthalmologic investigation to select animals with healthy eyes for this study. 58
203 animals for Montpellier and 33 animals for Hannover showing eye malformations were
204 thereby excluded from further analysis.

207 **Data analysis**

209 1. Manometric calibration of the IOP measured by TonoVet® and TonoPen™ in
210 enucleated eyes

211 For the manometric calibration of the TonoVet® and TonoPen™ IOP measurements, we
212 performed a regression analysis out of the eight means (= eight eyes) per step mmHg per
213 instrument.

215 2. Comparison of IOP between TonoPen™ and TonoVet®
216 For the in-vivo comparison of applanation (TonoPen™) and rebound (TonoVet®) tonometry,
217 we calculated the mean, range, standard deviation and median IOP for each instrument out of
218 the measurements of the 24 eyes of the 12 animals. For the TonoVet® tonometer and the
219 TonoPen™ tonometer, we compared measured IOP values between left and right eyes using
220 the paired t-test and measured IOP values between sexes using the unpaired t-test since the
221 values followed a normal distribution. The IOP values obtained by the TonoPen™ and
222 TonoVet® were compared using the Wilcoxon-signed-rank test.

224 3. Comparison of IOP across colonies

225 To assess the effect of sex and eye position, IOP values (using TonoVet®; $N_{\text{totalanimals}} = 258$,
226 $n_{\text{totaleyes}} = 516$; Hannover, $N = 75$; Montpellier, $N = 183$) obtained for each eye and animal per
227 colony were compared between the left and right eye and between sexes using the Wilcoxon-
228 signed-rank test and Mann-Whitney-U test, respectively.

229 If findings within colonies did not reveal a significant effect of eye position or sex, we used
230 the median value of an animal per colony for further statistical analysis. To explore the effect
231 of colony, we compared IOP values between colonies using the Mann-Whitney-U test.

232 The effect of age on IOP was analysed using a Spearman-Rank correlation. For the colony of
233 Montpellier, the cycle age was multiplied by the factor 1.5 to calculate the chronological age
234 in years. To define the reference IOP value for a healthy mouse lemur eye, we calculated the
235 mean, range, standard deviation and median of the IOP of the healthy eye of both colonies.

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Results

1. *In vitro calibration of the rebound (TonoVet®) and applanation (TonoPen™) tonometry*

We found a linear correlation between either the rebound tonometry and direct manometry or applanation tonometry and direct manometry (see Fig. 4; n = 8 eyes; N = 4 mouse lemurs). The regression analysis showed consistent linear underestimation of IOP by the TonoVet® and TonoPen™. From 15 mmHg up to 100 mmHg, the regression analysis showed that the measured IOP (mIOP) for TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) can be corrected by using the function $tIOP = 0.981 + (1.962 * mIOP)$ and for TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) by using the function $tIOP = 5.38 + (1.426 * mIOP)$. In both tonometers it was not possible to obtain values below 15 mmHg. From 15 mmHg up to 100 mmHg the TonoVet® constantly underestimated the tIOP by half. While the TonoPen™ almost measured the same values at 20 mmHg as the TonoVet®, the measured values start slightly to increase compared to the TonoVet® reaching around 66% of the tIOP at 100 mmHg.

2. *In-vivo IOP measurements with TonoPen™ and TonoVet®*

A comparison of IOP values between the left and right eye for each instrument showed no significant difference between eye side (TonoVet®, paired t-test, $N = 12$, $t = -0.538$, $p = 0.601$; TonoPen™, paired t-test, $N = 12$, $t = -0.794$, $p = 0.444$).

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3 273 No difference between sexes was found for IOP neither for the TonoVet® (unpaired t-test,
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5 274 $N_{\text{total}} = 24$, $N_{\text{males}} = N_{\text{females}} = 6$, $t = -0.130$, $p = 0.292$) nor for the TonoPen™ (unpaired t-test,
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7 275 $N_{\text{total}} = 24$, $N_{\text{males}} = N_{\text{females}} = 6$, $t = -0.340$, $p = 0.198$). Thus, we have not differentiated between
8
9 276 sexes in all further analyses.
10
11 277 The mean IOP for the TonoVet® tonometer was 9.21 ± 1.53 mmHg, the median was 9.0
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13 278 mmHg and the range 6-12 mmHg, the tIOP was 19.03 ± 2.24 mmHg.
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15 279 For the TonoPen™ tonometer the mean was 23.83 ± 5.89 mmHg, the median 24.5 mmHg
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17 280 and the range 14-38 mmHg with a tIOP of 39.36 ± 5.1 mmHg. (see Tab. 1)
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19 281 IOP values measured by the TonoVet® and the TonoPen™ differed significantly (Wilcoxon-
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21 282 test, $N = 24$, $T = 0.00$, $n = 24$, $p = < 0.001$). The comparison of measured values between
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23 283 instruments showed a higher estimation of the average IOP for the TonoPen™. The average
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25 284 value for the TonoPen™ is more than twice as high as the average value estimated by the
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27 285 TonoVet®. Measurements with the TonoPen™ in the same animal for the left and right eye
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29 286 show high variability, while the values obtained with the TonoVet® are much more
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31 287 consistent e.g. Peanut (TonoPen™ left eye 38 mmHg, right eye 16 mmHg; TonoVet® left
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33 288 eye 10 mmHg, right eye 9 mmHg). (see Fig. 5)
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41 290 Several problems occurred in the application of the TonoPen™ in the examiner-animal
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43 291 context. The tip of the TonoPen™ was large in comparison to the small eyes of the mouse
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45 292 lemurs causing reflective winks of the animal because of contact to the animals' eyelashes. It
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47 293 was not possible to fixate the animals' eye-lids without causing indirect pressure on the eye-
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49 294 bulb. Besides, the examiner had to protect himself against bites since the hand which was
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51 295 fixating the animals' eye-lids was hazardously close to the animals' mouth and sharp teeth.
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53 296 The time that was necessary to complete one successful measurement per animal varied
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55 297 between 5 and 10 minutes (without waiting time for the applied anaesthetizing eye-drops).
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299 When using the TonoVet® for IOP measurement, the animals showed no visible reaction

300 when the probe touched the animals' cornea, furthermore almost no reflective wink was

301 visible. Since no forced fixation of the animals' eye-lids was necessary, bites never occurred.

302 The investigation lasted on average only 30 seconds.

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305 *3. Comparison of IOP between two mouse lemur colonies and the definition of a*

306 *reference IOP value for the gray mouse lemur*

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308 Both colonies have been investigated using the TonoVet®. A comparison between measured

309 IOP values in the left and right eye for each colony showed no significant differences

310 (Hannover, Wilcoxon-test, $N = 75$, $T = 22.05$, $n = 47$, $p = 0.361$; Montpellier, Wilcoxon-test,

311 $N = 183$, $T = 55.27$, $n = 113$, $p = 0.336$).

312 No difference between sexes was found for IOP (Hannover, Mann-Whitney-test, $N_{\text{total}} = 150$,

313 $N_{\text{males}} = 37$ eye-pairs, $N_{\text{females}} = 38$ eye-pairs, $U = -1.314$, $p = 0.189$; Montpellier, Mann-

314 Whitney-test, $N_{\text{total}} = 366$, $N_{\text{males}} = 82$ eye-pairs, $N_{\text{females}} = 101$ eye-pairs, $U = -1.552$, $p =$

315 0.121). Thus, sexes were not further differentiated in further analyses.

316 The comparison of IOP values between both colonies showed no significant difference

317 (Mann-Whitney-test, $N_{\text{total}} = 516$, $N_{\text{Hannover}} = 75$ eye-pairs, $N_{\text{Montpellier}} = 183$ eye-pairs, $U = -$

318 0.230 , $p = 0.818$). Based on that, we did not further differentiate between colonies for

319 subsequent analysis.

320 The effect of age on IOP was assessed by a Spearman-Rank correlation. No significant

321 correlation between chronological age and IOP was revealed ($p = 0.418$, $r_s = -0.036$, $N = 516$,

322 see Fig. 6). Thus, age has not affected IOP in the healthy eye for the investigated age-span.

Based on that a reference value for IOP measured with the TonoVet® was calculated with a mean of 9.87 ± 1.56 mmHg, a median of 10 mmHg and a range of 5-15 mmHg. Using the regression function the calculated mean for healthy mouse lemur eyes is $tIOP = 20.3 \pm 2.85$ mmHg.

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Discussion

This is the first study measuring the IOP of mouse lemurs. Our findings suggest that the TonoVet® is the most suitable tool (compared to the TonoPen™) for rapid IOP screening of the tiny eyes of this smallest bodied primate. Results showed that IOP in the clinically healthy eye is not affected by age, sex, eye side (left or right). Thus, a reference value for IOP could be defined based on a large sample-size of more than 250 individuals for this novel primate model for aging research.

Calibration of IOP using manometry

Every tonometer has to be calibrated for each species specifically due to the fact of different corneal attributes, especially corneal thickness, to get the tIOP values. (33, 34) IOP measurements using eyes persisting in the eye socket in ex-vivo as well as enucleated eyes revealed no significant differences between these procedures. (35) Manometric in-vivo measurement of IOP is, however, known to potentially cause damage at intraocular structures and pain, thus for ethical reasons we decided to base our manometric investigation on enucleated eyes of animals which died for natural reasons or were euthanized due to incurable diseases which had no impact on IOP.

Our manometric calibration for the TonoVet® and TonoPen™ showed a consequent linear underestimation of the tIOP for both instruments which can be corrected by the established regression functions. For a fast clinical interpretation of the measurements displayed on the TonoVet® the values can be multiplied by 2. For the TonoPen™ a similar but not as easy to use formula is: $tIOP = (1.5 * \text{measured value}) + 5$.

A comparable underestimation of the IOP measured by the TonoVet® and TonoPen™ was reported for dog eyes. (15) This underestimation was explained by corneal specification

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3 373 especially corneal thickness, calibration-standards and use by different examiners. For our
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5 374 study, the same examiner performed the measurements so that the effect of the examiner on
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7 375 the measured IOP values was minimized whereas both corneal specification and different
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9 376 calibration standards between TonoVet® and TonoPen™ are likely to explain the
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11 377 underestimation of IOP values. In humans a significant effect on IOP measurements caused
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13 378 by different thickness in different corneal areas using the rebound tonometry has already been
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15 379 shown: higher values were determined when corneal thickness was higher. (33, 34) Our
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17 380 measurements were taken at the center of the cornea to minimize this effect. We expect that
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19 381 central corneal thickness in mouse lemurs is relatively thin, which would explain the linear
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21 382 underestimation. Further investigations on corneal thickness of mouse lemurs e.g. with an
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23 383 ultrasound pachymeter are necessary to investigate this in more detail.
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30 385 **Practicability of TonoPen™ or TonoVet® to screen the IOP of mouse lemur eyes**

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32 386 The practical value of a tonometer is as important as the calibration. Thus, which of the
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34 387 commonly used tonometers in veterinary science, TonoPen™ or TonoVet®, is the most
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36 388 suitable tool to screen tiny eyes of a large number of mouse lemurs in colonies on a regular
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38 389 basis? The eyes of the gray mouse lemur have only a diameter of 9.4 mm. (11) Thus, a
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40 390 previous ophthalmological study in the gray mouse lemur had difficulties in using
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42 391 applanation tonometry in non-anesthetized animals. (13) We applied both the TonoPen™ and
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44 392 the TonoVet® for a subgroup of non-anesthetized animals to assess the practicability of these
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46 393 instruments. We showed that both tonometers can be applied, but that there are huge
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48 394 differences in practicability and ethical justifiability. The investigation with the TonoPen™
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50 395 required a much longer time (up to 10 minutes) to assess the IOP of an animal compared to
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52 396 TonoVet® (up to 30 seconds). The most time consuming issue emerged by the high number
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54 397 of failed IOP measurements (indicated by an alarm-signal of the TonoPen™). The failure in
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3 398 measurement was due to the fact that the veterinarian had to pay attention to exerted pressure,
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5 399 contact-area and animal position while measuring the IOP. In contrast, unsuccessful
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7 400 measurements (indicated by an alarm-signal) were quite rare for the TonoVet®. Furthermore,
8
9 401 the small eye-bulbs of the mouse lemurs make it necessary to fixate the eye-lids manually
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11 402 and eventually causing pressure on the eye-bulb whereas no fixations of the animal's eye-lids
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13 403 were necessary for the TonoVet®. The animal itself must be fixated much stronger when
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15 404 using the TonoPen™ causing systemical hypertension and consecutively higher IOP.
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17 405 Consequently, our experience using the TonoPen™ supports Beltran et al. (13) that the use of
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19 406 this instrument to measure IOP in the tiny eyes of mouse lemurs is problematic. The
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21 407 extensive manipulation which is necessary prevents the determination of physiological
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23 408 expected IOP values. Usually physiological IOP ranges from 15-23 mmHg e.g. in humans,
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25 409 (36) dogs, (14) cats, (18) horses, (28) rabbits, (23) rats (21) and macaques. (37) (see Tab. 2)
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27 410 Therefore non-physiologically high IOP as measured with the TonoPen™ in mouse lemurs
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29 411 ($tIOP = 39.36 \pm 5.1$) may be the result of stress, high systemic blood pressure and unintended
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31 412 pressure on the bulbus questioning the ethical justifiability of this method.
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33 413 Other positive effects of the TonoVet® were that IOP of non-anesthetized animals can be
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35 414 measured rapidly and without any visible harm for the measured animal. Furthermore a
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37 415 veterinarian can standardize the measurement quickly and get fast routinisation. The
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39 416 TonoVet® showed satisfying results concerning reproducibility with a relatively small
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41 417 variation comparable to those of other studies using larger mammals such as rhesus macaques
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43 418 (25) or rabbits. (26) All in all, based on these findings we recommend the TonoVet® as a
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45 419 suitable IOP assessment tool for rapid screening of the eyes in non-anaesthetized mouse
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423 Definition of a reference IOP for the healthy gray mouse lemur eye

424 A reliable mean for IOP in mouse lemurs requires a large sample-size. To enlarge our
425 sample-size we have analyzed if eye side (left or right), sex or age had any influence on IOP
426 in mouse lemurs. Our study showed no significant differences between eye side and sex and
427 no statistically significant correlation between age and IOP. The slight decrease of IOP
428 between the age of 0.5 and 10 years was even smaller than the value of the determined
429 standard deviation. Animals of ten years and older were excluded due to diagnosed
430 pathologies.

431 Our screening analysis of the two colonies, Hannover and Montpellier, included 258 animals
432 with 516 healthy eyes in total and showed a tIOP of 20.3 ± 2.85 mmHg. This value matches
433 quite well to the IOP range of 15-23 mmHg reported from humans (36) and mammals of
434 veterinary and biomedical importance (see Tab. 2) such as dogs, (14) cats, (18) horses, (28)
435 rabbits, (23) rats (21) and macaques. (37) Since these mammals differ largely in size, activity
436 and phylogeny, IOP seems to be independent from these factors.

437 Circadian rhythm is also described to affect IOP, e.g. in cats, rabbits and Tibetan monkey.
438 (23),(38),(39) To minimize this effect, we always determined IOP at the beginning of the
439 animal's activity period.

440 Our study showed no correlation between age and IOP in the healthy mouse lemur eye
441 comparable to e.g. Tibetan monkeys. (39) Age-effects on IOP in animals and humans are
442 ambiguous. Whereas investigations performed in rhesus monkeys and dogs showed a
443 decrease in IOP with age (37),(14) studies in humans revealed both an increase and a
444 decrease depending on the tested populations. (40),(36),(41) High blood pressure, obesity and
445 other vascular deficiencies were discussed as explanations. (42-44) Consequently we
446 postulate that physiological status and vascular conditions were healthy in our studied mouse
447 lemur population.

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448 It has to be taken into account that for our study we considered only ophthalmological
449 inconspicuous animals. Pathologies influencing the state of health of the eye and leading to
450 glaucoma or higher intraocular pressure have to be considered in follow-up studies.

451
452 Conclusion

453 To conclude, we demonstrated the practicability, usefulness and reliability of the TonoVet®
454 as a powerful tool for screening IOP in mouse lemurs, a novel primate model for human
455 aging research. Average IOP of healthy mouse lemur eyes is not affected by eye side, sex and
456 colony and does not correlate with age. Furthermore, the value of IOP of mouse lemurs
457 coincides with those of other mammals. For future studies using this smallest-bodied primate
458 aging model, our findings are an important foundation to disentangle peripheral from central
459 pathologies.

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463 Software for statistical analysis

464 The statistical analysis was performed using SPSS 22.0 for Windows. Significance level was
465 set at $P = 0.05$.

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468 List of Abbreviations

469 IOP: Intraocular pressure; tIOP: true intraocular pressure; mIOP: measured intraocular
470 pressure

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472 Competing interests

473 The authors declare that they have no competing interests.

474

475 Authors' contributions

476 MD, MJ, EZ, IN, JS have conceived, coordinated and designed the study. Manometrical data

477 was acquired by MD. Data from the screening of both colonies was acquired by MD and MJ.

478 Statistical analysis was conducted by MD. All authors contributed in drafting, reading and

479 approving the final manuscript.

480

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618 **Figure 1. Setup of the manometrical investigation.**

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620 **Figure 2. Investigation of a 2 year old mouse lemur with the TonoVet®.**

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622 **Figure 3. Investigation of a 2 year old mouse lemur with the TonoPen™**

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624 **Figure 4. Values measured with the TonoVet® and TonoPen™ under manometrical**
625 **control.** Manometrically determined IOP for the eight eyes of four animals are represented
626 on the x-axis. TonoVet® and TonoPen™ values are represented on the y-axis. Each dot
627 represents the mean from three measurements per step/per eye. The dashed line represents the
628 regression line for the TonoVet® ($F = 28263.232$, $r^2 = 1$, regression analysis, $p < 0.001$) and
629 TonoPen™ ($F = 3497.514$, $r^2 = 0.997$, regression analysis, $p < 0.001$) measurements.

630

631 **Figure 5. Comparison of the in-vivo measured IOP for the TonoVet® and TonoPen™ in**
632 **12 animals.** The results show high variation in measurements for the TonoPen™ and much
633 more consistent values for the TonoVet®. E.g. Peanut (TonoPen™ left eye 38 mmHg, right
634 eye 16 mmHg; TonoVet® left eye 10 mmHg, right eye 9 mmHg). For exact values (see Tab.
635 1).

636

637 **Figure 6. Relation between tIOP values and age in healthy mouse lemur eyes.** This
638 scatterplot shows the tIOP values for all measured healthy animals from Hannover and
639 Montpellier ($N = 258$) on the y-axis in relation to age on the x-axis. The decrease in IOP is
640 statistically not significant ($p = 0.077$, $r_s = -0.110$).

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Table 1. Overview of intraocular pressure (IOP) values in 12 animals for TonoVet® and TonoPen™.

The median, range, mean and standard deviation have been calculated for each instrument using the median from each animal’s right and left eye.

	TonoVet® IOP results (mmHg)		TonoPen™ IOP results in mmHg	
Animal name	Right eye	Left eye	Right eye	Left eye
Pixel ♂ (2 years)	10	9	27	22
Quilla ♀ (3 years)	11	11	21	29
Quinn ♂ (3 years)	9	10	18	25
Quelle ♀ (3 years)	9	8	24	28
Quirl ♂ (3 years)	8	7	17	16
Pippi ♀ (2 years)	10	6	22	14
Paloma ♀ (2 years)	10	9	23	25
Peanut ♂ (2 years)	9	10	16	38
Quentin ♂ (3 years)	11	11	28	18
Queenie ♀ (3 years)	8	12	19	33
Pandora ♀ (2 years)	7	10	27	26
Phil ♂ (2 years)	7	9	31	25
Median (mmHg)	9		24.5	
Range (mmHg)	6-12		14-38	
Mean ± SD (mmHg)	9.2 ± 1.53		23.83 ± 5.89	

Table 2. Variation of calibrated intraocular pressure (IOP) values in different species

Species	Reference	Mean IOP or range	Standard deviation
Human (N > 100.000)	[43]	15-16 mmHg	± 2.5-3 mmHg
Macaques (N= 102)	[44]	14.9 mmHg	± 2.1 mmHg
Gray mouse lemur (N = 516)	this study	20.3 mmHg	± 2.85 mmHg
Cats (N = 57)	[29]	20.74 mmHg	± 0.49 mmHg
Horses (N = 35)	[39]	22.1 mmHg	± 5.9 mmHg
Rabbits (N = 38)	[34]	15-23 mmHg	
Lewis rats (N = 115)	[32]	17.3 mmHg	± 5.25 mmHg
Dogs (N > 900)	[25]	19.0 mmHg	± 5.7 mmHg