Measurements of Three Ocular Parameters in the Göttingen Minipig

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Summary

The use of laboratory pigs has increased dramatically in the last decade, and this study supplements the basic ocular anatomical and physiological characterisations already carried out on laboratory pigs. Pigs are frequently used as models of human ocular diseases due to the similar anatomy and physiology of the ocular structures of the pig as compared to humans, but refractive error has not been investigated systematically in the Göttingen minipig. We measured refractive status, corneal power and axial length in a sample of 30 adult Göttingen minipigs including both sexes. The uncorrected mean refractive error was +1.3 dioptres (D) with a standard deviation (SD)± 2.3 D.The mean corneal power was 44.1 D (SD±1.5D) and mean axial length less than 19 mm. No statistically significant difference was detected between the right and left eye values, with respect to colour of iris, or between genders ($p<0.05$). In spite of the smaller axial length, measures of refractive error and corneal power in the Göttingen minipig are comparable to human values. This information should prove useful when using Göttingen minipigs as models for human ocular conditions or in research involving vision or other ophthalmic aspects of the Göttingen minipig. Also, the refractive status of the animal can be of importance when using pigs in cognitive tasks, mainly because of the probable lack of accommodative reflex in pigs. If visual stimuli are presented at a short distance, clinical, emmetropia or myopia in the experimental animals would be desirable; in the present cohort of examined pigs, 60% fulfilled these criteria.

Introduction

The use of various breeds of pigs for research purposes has increased during the last decades. Due to the similarities in anatomy and physiology of the ocular structures of the pig as compared to humans (*Prince et al., 1960; Gerke et al., 1995; Vilipuru & Glasser, 2001; Zeng et al., 2001*), pigs are often used as models of human ocular diseases (*Shih et al., 1998; Petters et al., 1997; Sweatt et al., 1999; Andreo et al., 1999; Garcia-Layana et al., 1997; Steinemann et al., 1998; Mahmoud et al., 2003; Knudsen et al., 2001*). Pigs have also been used in studies of memory and learning (*Moustgaard et al., 2002; Moustgaard et al., 2004*), but when using pigs in visually guided tasks, knowledge about the porcine vision is important. The literature on the porcine vision is sparse, but refractive error has been measured several times ex-vivo as part of the evaluation of refractive correctional techniques (*Sobol et al., 2002; Koopmans et al., 2003*); and several ocular parameters have been measured in a Taiwanese laboratory pig breed, Lee-Sung pigs (*Shih et al., 1998*). The most frequently used laboratory pig in Europe is the Göttingen minipig, and

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although spontaneous ocular findings have been reported in this species (*Loget, 1995*), specific ocular parameters of importance to vision have not been investigated in the Göttingen minipig so far.

Emmetropia is important for normal vision, and is found when parallel rays are refracted to focus upon the retina. In the human population emmetropia is described by Sorsby (1979) "as a point in the modal range which extends from 0 to +2 dioptres (D)", the most common value being around + 0.75 D (*Sorsby, 1979*). Several other classification systems exist though, and emmetropia is therefore not distinctly defined and varies among investigators (*Weymouth & Hirsch, 1991*). A refractive error, however, is found when parallel rays of light are not focused upon the retina, but are focused in front or behind it. Refractive errors are the most common cause of poor vision in man. The refractive status of the eye is influenced by the diameter of the globe (axial length), corneal power (keratometry) (*Sorsby et al., 1957*), anterior chamber depth and lens power (*Goss & Erickson, 1990; Garner et al., 1992*). There does not exist a simple correlation between these factors, but there is proportionality between axial length (and lens power) and degree of myopia (*Koretz et al., 1995; Garner et al., 1992*), while the relationship between refractive error and corneal power is not a consistent finding.

In order to obtain normal values of the refractive error in the Göttingen minipig, we measured the refractive error in adult Göttingen minipigs and additionally two parameters (corneal power and axial length) contributing to vision.

Materials and Methods

The study comprised 18 male and 12 female Göttingen minipigs (Ellegaard Göttingen Minipigs ApS, Soroe Landevej 302, DK-4261 Dalmose, Denmark) of age 6-11 months, health monitored according to Federation of European Laboratory Animal Science Associations (FELASA) guidelines (revealing only rotavirus) (*Rehbinder et al., 1998*). The examination took place partly at Ellegaard Göttingen Minipigs and partly at the Royal Veterinary and Agricultural University, Copenhagen. All animals were housed and cared for according to the principles of the Danish Animal Experimentation Act (based on the Council of Europe Convention ETS 123).

Approximately thirty minutes before the examination, the pupils were dilated with one drop of atropine sulfate (Atropin "PS", 1%, Pharma-Skan ApS, Skanderborg, Denmark). Measurements of cycloplegic refraction and keratometry (corneal power) were obtained by autorefraction (Retinomax, Nikon Instruments Europe B.V., Badhoevedorp, Netherlands) using infrared light at a distance of 5-10 cm from the eye. The autorefraction gives a quick, reliable measurement with high accuracy and reproducibility (*Salvesen & Kohler, 1991; Harvey et al., 1997; Harvey et al., 2000*). At least five measurements of the cycloplegic refraction were made, and the reported values are the autorefractor-calculated median value. The keratometric values were recorded by the device as up to eight separate estimates of corneal curvature along two meridia, 90° apart. The mean value along each meridian was determined, and the average of the greater and lesser curvature represents the reported values of corneal curvature. An assistant manually prevented closure of the eyelids by pressing it gently towards the bony construct of the upper and lower rim of the orbital cavity and thereby avoiding applying pressure on the globe. The axial length was measured by ultrasound with a Bio-Pen™ XL (Medtronic Solan, Chicago, U.S.A.) immediately after applying one drop of Oxybuprokain in each eye (Oxybuprokain "SAD", 0.4 %, Nycomed Danmark, Roskilde, Denmark). The tip of the Bio-Pen was placed on the central cornea in a 90° angle from the corneal surface in order to obtain the correct axis through the eye. The colour of iris was noted at the end of the examination. All examinations were performed on non-anaesthetized animals, restrained either manually or in appropriate slings. It was not possible to obtain measures from both eyes on every animal due to resistance to the examination.

The refractive errors are given as the spherical equivalent in dioptres (D). The keratometry values are given in dioptres (D), and are the mean of as many as possible horizontal and vertical measurements. Axial length measurements are reported in millimetres and are the mean of at least two consecutive ultrasonic measurements. The iris colour was denoted as either "blue" or "brown", with blue representing the colours light blue, grey blue and dark blue, and corresponding to classification type BB by von Wegner (*von Wegner, 1973*). All other colours of iris were in this experiment were denoted as "brown" corresponding to type AA and type AB iris colour in the that classification system.

Data analysis

The data set was evaluated for normality using the Kolmogorov-Smirnov normality test. Distributions were characterized as normal in the cases where the hypothesis of normality could not be rejected at $p < 0.05$. If the ocular values diverged to such an extent that it was reasonable to characterize the eye as having a developmental anomaly, the data were discarded from further analysis. The Retinomax automatically calculated the refractive error. The exclusion criterion of refractive error was a value differing more than 10 D from the mean/median, whereas for corneal power it was a value differing more than 6 D from the mean/median. Since the axial length to a very large extent is eye age-dependent (*Bartholomew et al., 1997*), even for adult pigs, there is no upper limit leading to exclusion. The lower limit of the Bio-pen, however, was 18.6 mm. For refractive errors and corneal power the statistical significance between right and left eye values was assessed using a paired t-test, while the statistical significance between the two sexes and between blue and brown eyes, respectively, was assessed using Student's t-test. Since the values obtained from the two eyes cannot be regarded as independent samples, the statistics were calculated from a mean value from the pairs of eyes in which values from both eyes were present. In those cases from which data was available from only one eye, that value was used in the analysis.

All data analysis was made using MINITAB 12.1 Statistical Software (Minitab Inc. State College, Pennsylvania, U.S.A.)

Results

The obtained measures of refractive errors ranged from –13 D to + 4.5 D, with a median of + 1.5 D. According to the exclusion criteria, the eye having a refractive error of –13 D was discarded from further analysis, since the condition probably reflects a structural developmental anomaly of that particular eye, and consequently cannot be regarded as representing the normal refractive error in Göttingen minipigs. Accordingly, refractive errors were normally distributed with a mean of +1.3 D and SD of ±2.3 (Figure 1). The anisometropia (interocular variation between the eyes in each pig) from 25 pigs ranged from 0.0 to 3 D with a median of 0.75 D (Figure 2). 74 % of the examined pigs could be categorized as having a clinically insignificant anisometropia, since its magnitude was below or equal to 1 D. 26% of the examined pigs were anisometropic between 1.5 D and 3 D. Of the examined pigs, where data of refractive error were present from both eyes, 48% were emmetropic in both eyes, and 60% were either emmetropic or myopic in one or both eyes. Hence, 40% of the examined pigs

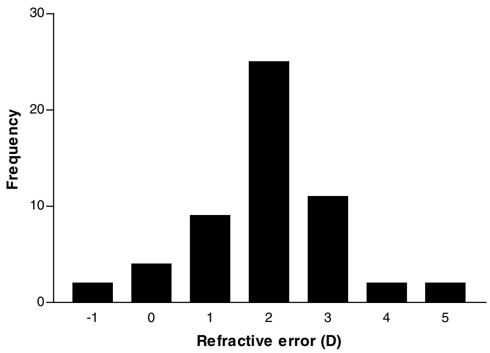

Figure 1. Frequency distribution of measurements of refractive errors (in dioptres) of 55 eyes from 30 adult Göttingen minipigs.

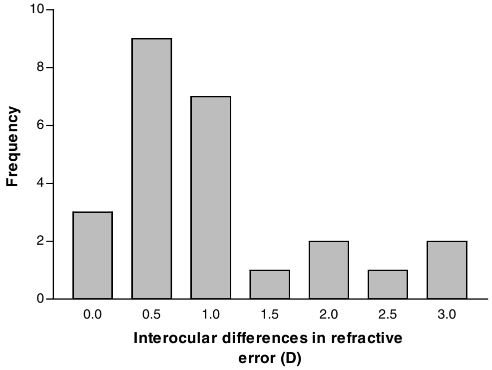

Figure 2. Frequency distribution of the interocular differences (right-left) in refractive error (in dioptres) of 25 adult Göttingen minipigs.

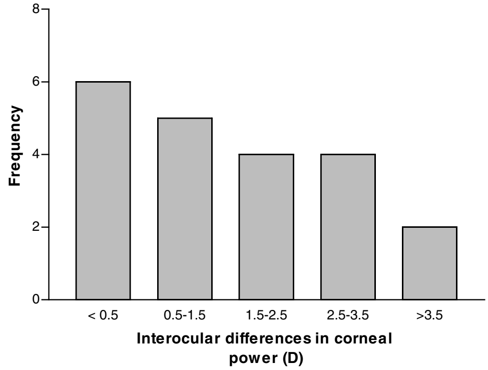

Figure 4. Frequency distribution of the interocular differences (right-left) in corneal power (in dioptres) of 21 adult Göttingen minipigs.

were hyperopic in at least one of the eyes and of these 40% had anisometropia > 1D. There were not statistically significant differences in refractive error between right and left eye values, or between genders.

Corneal power, ranging from 40 to 47.3 D, was normally distributed with a mean 44.1 D and SD ±1.5 D (Figure 3). The interocular variation in corneal power from 21 pigs ranged from 0.0 to 4 D with a median of 0.87 D (Figure 4). There were not statistically significant differences in corneal power between the right and left eye values, or between genders.

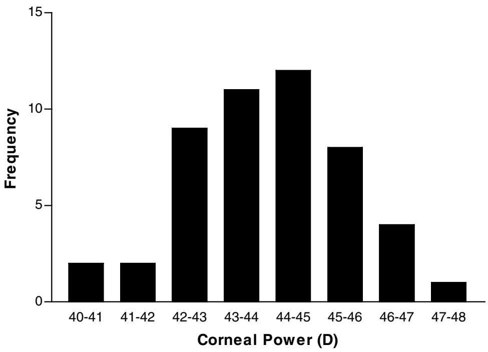

Figure 3. Frequency distribution of measurements of corneal power (in dioptres) of 51 eyes from 30 adult Göttingen minipigs.

For a majority of the Göttingen minipigs (56%) examined, the axial length was below 19 mm (Table 1).

There was no apparent relationship between refractive error and corneal power (r=0.3, p=NS) (data not shown). It was not possible to establish any correlation to axial length due to the nature of these data.

47 % of the pigs were characterized as having blue iris colour, while 50 % of the examined pigs were characterized as having the iris colour brown. Heterochromacity was present in 3 % of the examined pigs. Any statistical significance of the three parameters in relation to colour of iris could not be established.

Table 1. Frequency distribution of axial length (in mm) of 44 eyes from 24 adult Göttingen minipigs.

Axial length (mm)		
< 19	19-20	> 20
25	16	3

Discussion

We measured refractive error in a population of 30 adult Göttingen minipigs and two other ocular parameters of importance to vision.

Measures of refractive error were obtained by autorefraction using a handheld refractometer (Retinomax®), which at the same time measured the corneal power. This method allows quick reliable measures of refractive error and corneal power in living, awake, and non-sedated pigs. Further, a great advantage of this method is that only a very brief period of visual fixation is necessary (less than 1 sec), since most pigs do not tend to fixate on the Retinomax for longer. The pig, however, should be able to fixate on the small object shown inside the instrument at least five times in order to make a reliable measure. Fixation is identified by the investigator as a location of four small dots in the centre of the dilated pupil, which ensures that the measures are indeed obtained while the pig is fixating.

The estimated mean refractive error in adult Göttingen minipigs was +1.3 D (SD±2.3 D), which is in accordance with reported measures of refractive error in Lee-Sung pigs (*Shih et al., 1998*). Also, it corresponds to human values, where the distribution of refractive error has a leptokurtic distribution with an average of approximately 1 D of hyperopia (*Sorsby, 1979*).

We found the mean axial length to be less than 19 mm. Bartholomew and colleagues (1997) reported a mean axial length of 21.64 mm in slaughter pigs, and Lee-Sung pigs have an axial length between 16 and 17 mm. (*Shih et al., 1998*). This agrees with the differences in adult body weight of the three breeds: Göttingen minipigs have larger body weight than Lee-Sung pigs, and considerably less than breeds used in meat production. The estimated mean axial length of humans is 23.94 mm (*Sorsby et al., 1957*), and hence larger than in pigs. For obtaining measures of axial length we used ultrasound by means of a Bio-Pen™. This instrument, however, was designed for use on human patients and had a lower limit of 18.6 mm, which was inconvenient since the axial length for a subpopulation of Göttingen minipigs is less than this. In addition, the method required physical contact between the pen and cornea; a procedure, which was not tolerated by all pigs in spite of the application of a local anaesthetizing agent to the cornea's surface.

The recorded mean corneal power was 44.1 D, which is close to the human value of 43-44 D (*Sorsby et al., 1957; Goss & Jackson, 1995; Tron, 1940*). Lee-Sung pigs, however, have considerably higher corneal power (56.6 D) (*Shih et al., 1998*), which, nevertheless, agrees with their smaller mean axial length (*Francois & Goes, 1977; Koretz et al., 1995*).

It was not possible to establish significant correlation between the measured ocular parameters. In dogs, correlations between refractive error and corneal power or axial length were net established, but refractive error correlated negatively with age (*Murphy et al., 1992b*), as it does in humans (*Grosvenor, 1987*). This phenomenon is also demonstrated in rhesus monkeys (*Bradley et al., 1999*). We used young adult minipigs of approximately the same age, so the effect of age on the ocular parameters was not investigated. It is possible that this is also true for Göttingen minipigs, but further studies are needed to elucidate this.

In the present study we only measured two parameters with implication for vision. Since anterior chamber depth and crystalline lens thickness also influence the measure of refractive error (*Goss & Erickson, 1990*), as do other parameters such as crystalline lens power (*Garner et al., 1992*), these should also be measured in order to give a more thorough description of the factors affecting the refractive status in pigs. Although not possible in the present study, as the examinations had to be carried out on awake, unsedated pigs, further studies of the relationship between the different ocular parameters, and the effect on age upon these, is to be encouraged.

We did not find evidence for a gender difference in the three ocular parameters measured in this population of 30 pigs. Among humans, women have shorter axial length and larger corneal curvature than do males, but there is no gender difference related to refractive error (*Midelfart, 1996; Wong et al., 2001*).

The practical implication of the findings on refractive error in relation to testing Göttingen minipigs in visually guided learning tasks is important. Firstly, if the stimuli in the experimental set-up are presented at a short distance (i.e. less than 50 cm.), the pigs should optimally be emmetropic or mildly myopic in order to see the stimuli clearly. In this study, 40% of the examined minipigs were not. In humans, accommodation, which is the process by which the eye changes its refractive power to focus on near objects by contraction of the ciliary muscle, can partly compensate hyperopia. However, preliminary studies showed that the refractive error did not change after topical administration of 1% atropine, indicating that the Göttingen minipig, in line with findings in other vertebrates (*Murphy et al., 1992b; Murphy et al., 1992a; Kendrick, 1990*), have a limited or non-existent accommodative ability. Consequently 40% of the minipigs will be expected to perceive stimuli presented on a short distance, as for example in cognitive tasks, as blurred. An investigation of the refractive error of pigs prior to experiments involving vision could be beneficial.

Here, we found an almost equal distribution of blue and brown coloured irises (47% and 50% respectively). An earlier study on Göttingen minipigs showed that only 13-17% of the examined groups of pigs had blue irises (*Loget, 1995*). Likewise, in German landrace pigs 15 % had blue irises (*von Wegner, 1973*). We found heterochromacity in 3% of the pigs, which is less than former findings in Göttingen minipigs (11-40%) (*Loget, 1995*). In other strains of minipigs the reported frequency of (bilateral) heterochromacity is 9% (*Gelatt et al., 1973*). The discrepancies between the distribution of iris colour and heterochromacity in our study and the former study on Göttingen minipigs could partly be due to the smaller sample size in our study, but possibly also that the breeding programmes since 1995 have been in favour of blue irises. The supplier of the minipigs was the same in both studies.

Several animal models of various structural ocular anomalies exist. The closer evolutionary distance between monkeys and humans have made monkeys a popular animal model for studying eye development, myopia and various other ocular conditions (*Bradley et al., 1999; Raviola & Wiesel, 1990; Smith et al., 1987; Iuvone et al., 1991; Repka & Tusa, 1995*), while pigs are often superior to other species in surgical studies due to similarities of functional anatomy and/or practical reasons (*Shih et al., 1998; Sweatt et al., 1999; Andreo et al., 1999; Steinemann et al., 1998; Garcia-Layana et al., 1997; Mahmoud et al., 2003; Knudsen et al., 2001*). However, since the use of monkeys in experimental research for several reasons are difficult and by some ethically questioned, it is likely that in the future pigs will serve more often as animal models for human ocular conditions. Our findings support Göttingen minipigs as likely candidates for future use in this regard and also supplements the basic ocular anatomical and physiological characterisations carried out on laboratory pigs (*Loget, 1995; Saint-Macary & Berthoux, 1994*) in order to compliment the increased replacement of monkeys and dogs with pigs in toxicology.

Furthermore with the progress of using genetically engineered pigs (*Petters et al., 1997; Mahmoud et al., 2003*) knowledge of standards of various ocular parameters might be beneficial.

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