Computed tomography analysis of guinea pig bone: Architecture, bone thickness and dimensions throughout development

The domestic guinea pig, *Cavia aperea f. porcellus,* belongs to the Caviidae family of rodents. It is an important species as a pet, a source of food and in medical research. Adult weight is achieved at 8-12 months and life expectancy is ~5-6 years. Our aim was to map bone local thickness, structure and dimensions across developmental stages in the normal animal. Guinea pigs (n=23) that had died of natural causes were collected and the bones manually extracted and cleaned. Institutional ethical permission was given under the UK Home Office guidelines and the Veterinary Surgeons Act. X-ray Micro Computed Tomography (microCT) was undertaken on the left and right scapula, humerus and femur from each animal to ascertain bone local thickness. Images were also used to undertake manual and automated bone measurements, volumes and surface areas, identify and describe nutrient, supratrochlear and supracondylar foramina. Statistical analysis between groups was carried out using ANOVA with post-hoc testing. Our data mapped a number of dimensions, and mean and maximum bone thickness of the scapula, humerus and femur in guinea pigs aged 0-1 month, 1-3 months, 3-6 months, 6 months-1 year and 1-4 years. Bone dimensions, growth rates and local bone thicknesses differed between ages and between the scapula, humerus and femur. The microCT and imaging software technology showed very distinct differences between the relative local bone thickness across the structure of the bones. Only one bone showed a singular nutrient foramen, every other bone had between 2 and 5, and every nutrient canal ran in an oblique direction. In contrast to other species, a supratrochlear foramen was observed in every humerus whereas the supracondylar foramen was always absent. Our data showed the bone local thickness, bone structure and measurements of

guinea pig bones from birth to 4 years old. Importantly it showed that bone development continued after 1 year, the point at which most guinea pigs have reached full weight. This study is the first to show the high abundance (100% in this study) of the supratrochlear foramen within the guinea pig humerus and the complete absence of a supracondylar foramen which is different to many other species and may also affect potential fracture points and frequencies. Understanding bone morphology and growth is essential in not only understanding the requirements of the healthy guinea pig, but also necessary in order to investigate disease states.

1 **Computed Tomography Analysis of Guinea Pig Bone: Architecture, Bone Thickness and**

2 **Dimensions throughout Development**

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Abstract

The domestic guinea pig, *Cavia aperea f. porcellus,* belongs to the Caviidae family of rodents. It is an important species as a pet, a source of food and in medical research. Adult weight is achieved at 8-12 months and life expectancy is ~5-6 years. Our aim was to map bone local thickness, structure and dimensions across developmental stages in the normal animal. Guinea pigs (n=23) that had died of natural causes were collected and the bones manually extracted and cleaned. Institutional ethical permission was given under the UK Home Office guidelines and the Veterinary Surgeons Act. X-ray Micro Computed Tomography (microCT) was undertaken on the left and right scapula, humerus and femur from each animal to ascertain bone local thickness. Images were also used to undertake manual and automated bone measurements, volumes and surface areas, identify and describe nutrient, supratrochlear and supracondylar foramina. Statistical analysis between groups was carried out using ANOVA with post-hoc testing. Our data mapped a number of dimensions, and mean and maximum bone thickness of the scapula, humerus and femur in guinea pigs aged 0-1 month, 1-3 months, 3-6 months, 6 months-1 year and 1-4 years. Bone dimensions, growth rates and local bone thicknesses differed between ages and between the scapula, humerus and femur. The microCT and imaging software technology showed very distinct differences between the relative local bone thickness across the structure of the bones. Only one bone showed a singular nutrient foramen, every other bone had between 2 and 5, and every nutrient canal ran in an oblique direction. In contrast to other species, a supratrochlear foramen was observed in every humerus whereas the supracondylar foramen was always absent. Our data is the first to show the bone local thickness, bone structure and measurements of guinea pig bones from birth to 4 years old. Importantly it showed that bone development continued after 1 year, the point at which most guinea pigs have reached full weight. This study is the first to show the high abundance (100% in this study) of the supratrochlear foramen within the guinea pig humerus and the complete absence of a supracondylar foramen, which is different to many other species and may also affect potential fracture points and frequencies. Understanding bone morphology and growth is essential in not only understanding the requirements of the healthy guinea pig, but also necessary in order to investigate disease states.

Introduction

The domestic guinea pig (*Cavia aperea f. porcellus*) belongs to the Caviidae family of rodents (Burnie 2008) that includes subfamilies covering species closely related to guinea pigs such as the Patagonian cavy, also known as the Mara *(Dolichotis patagonum)* or the world's largest rodent, the capybara (*Hydrochaerus hydrochaeris*)(Burnie 2008). The guinea pig has been kept as an important source of food, and is still eaten in many parts of South America, Asia and Africa (Meredith & Redrobe 2010; Morales 1995; NRC 1991). Peru alone has around 20 million guinea pigs, providing around 17 thousand tonnes of meat per annum, just 4,000 tonnes less than their sheep meat production (NRC 1991). The meat can fetch higher prices than pork or beef on small mountain farms in such regions as Ecuador (NRC 1991). It has a relatively high protein and low fat content in comparison to other alternatives such as chicken, which makes it a good nutritional enrichment for many lower socio-economic families (Numbela & Valencia 2003).

Guinea pigs are commonly kept as pets, as well as used extensively in medical research playing a pivotal role in epidemiological study and pharmaceutical development (Terril & Clemons 1997). The population of guinea pigs used in research has declined from 2.5 million in the 1960's to just over 200,000 in 2010 (Gad 2013; USDA 2011), but the popularity of the guinea pig as a pet has soared. The number of guinea pigs kept as pets in the UK has consistently been estimated at between 0.5 and 1 million since 2009, with the guinea pig presently listed as the UK's 8th most popular pet and highest ranking rodent (PFMA 2013).

Adults can achieve their full weight of up to 1800 grams but more usually between 900-1200 grams for boars and 700-1100 grams for sows (Behrend 2008; Meredith & Redrobe 2010) at 8-12 months, a higher weight than that of wild guinea pigs (Hubrecht & Kirkwood 2010), however these values vary greatly across the literature and can only be used as a rough guide. As a hystricomorph, the guinea pig sow has a characteristically long gestation period of 59 to 72 days, approximately double that of the rabbit (Meredith & Redrobe 2010). Litter size varies from 2 to 6, with an average of three or four pups, each weighing between 40 and 120 grams (Terril & Clemons 1997). The precocial pups are born mobile, fully-furred, with their eyes open and teeth present, and are therefore able to consume solid food within a few hours, although still suckle for two to three weeks (Hubrecht & Kirkwood 2010; Meredith & Redrobe 2010). Although there is wide variability across the literature, it is believed that puberty is reached at around six weeks in sows and between 9-10 weeks in boars (Harkness et al. 2010). Life expectancy is generally

72 considered to be between around 5-6 years (Mitchell & Tully 2009), although life expectancies of
73 up to eight years are reported (Gad 2013).

74 Amongst their many other functions such as mineral storage or bone marrow production, bones
75 are the main levers in mammalian bodies (Bilezikian et al. 2008) enabling the animal to move.
76 Bones have an additional secondary role in homeostasis as a store of calcium and phosphorus.
77 The bone is first formed as cartilage and becomes mineralised during the final stages of
78 pregnancy, a process that continues through to puberty and beyond, suggesting that the skeleton
79 of an older animal is able to withstand higher forces than that of a neonate (Sjaastad et al. 2010).
80 The bone consistency changes constantly throughout life as it undergoes remodelling in response
81 to physical and metabolic factors which can affect density as well as volume (Frost 1997;
82 Sjaastad et al. 2010).

83 Despite the significance of the guinea pig within the food and pet industry there has been little
84 research into the normal bone structure and density/thickness. One method that enables analysis
85 of bone characteristics is Computed Tomography (CT). Since its invention in the 1970's
86 (Hounsfield 1973), CT is now widespread in clinical imaging, permitting non-destructive and
87 non-invasive quantitative measurements of the body of both humans and animals. The imaging
88 technique is based on the attenuation of x-rays as they penetrate the material of interest at a
89 known number of angular positions. Subsequently, tomographic reconstruction algorithms are
90 used to generate a three dimensional spatial map of x-ray attenuation of a material which can then
91 be analysed in detail using computer software (Ritman 2011). Within the last 10 years, microCT
92 systems have become more common for non-clinical applications and offer higher spatial
93 resolution, detail detectability and contrast compared to conventional CT (Metscher 2009). The
94 technique has been successfully applied to the investigation of bone development and anatomy in
95 a range of small animals including the guinea pig, mouse and rat (Bialek et al. 2014; Tao et al.
96 2014; Uzun et al. 2007; Willett et al. 2012).

97 Long bones are composed of an outer layer of dense compact bone and an inner meshwork of
98 trabecular bone, which is particularly abundant in the epiphyses, and bone marrow (Zoetis et al.
99 2003).. In comparison, flat bones, such as the scapula, consist of two thick layers of compact
100 bone with a layer of trabecular bone in between. The blade of the scapula is said to undergo
101 intramembranous ossification, in which the bone develops from a fibrous membrane, whereas

102   some of the outer parts undergo endochondrial ossification whereas long bones such as the

103   humerus and femur form via endochondral ossification, where cartilage is replaced by bone (Ross

104   & Pawlina 2011; Scheuer et al. 2000). Regardless of its method of formation, bone growth in

105   length happens via cartilaginous growth plates, which fuse in later life, and in diameter by

106   periosteal apposition, enabling the bone to withstand increasing loads (Ross & Pawlina 2011).

107   Density can also vary throughout the bone, with the shaft of long bones, for example, having a

108   greater density than their extremities (Stiner 2004).

109   Our study investigated the bone growth and localised thickness of the guinea pig scapula,

110   humerus and femur. The rate of growth and the localised thickness are not known for these bones,

111   despite the frequency at which guinea pigs present in veterinary clinics with broken/fractured

112   limb bones. The scapula, along with the humerus, forms the shoulder joint. Similarly to the cat,

113   but unlike other domestic species, the scapula has a small clavicle attaching it to the manubrium

114   sterni. The scapula is a triangular shape and divided by a spine that runs over its lateral surface,

115   into a supraspinous and infraspinous fossa (Dyce et al. 2010). The distal part of the scapula ends

116   in the glenoid cavity, which serves as a surface for articulation with the head of the humerus,

117   forming the shoulder joint (Dyce et al. 2010). The humerus is comprised of the head,

118   contributing to the shoulder joint, and the articular condyle, part of the elbow joint (Dyce et al.

119   2010). Other notable features include the greater and lesser tubercle, the medial and lateral

120   epicondyles and the olecranon fossa (König & Liebich 2014).   The femur articulates with the

121   pelvis via its head forming the hip joint, and caudally with the tibia via its condyles to form the

122   stifle joint (Dyce et al. 2010). It is the strongest long bone of the skeleton and comprises the

123   proximal part of the hind limb with notable features including the greater and lesser trochanter

124   and the trochanteric fossa (König & Liebich 2014). This study investigates the attributes and

125   growth of three key limb bones, the scapula, humerus and femur. Clinicians and health care

126   advisors alike frequently highlight the importance of not dropping the guinea pig due to the

127   number of limb fractures and breaks that they observe in clinic (Richardson 2003). This study

128   aims to increase the information known about the limb bones, whilst showing how and when

129   development is occurring, in addition to providing localised bone thickness information.

130 The aims of this study were to utilise microCT technology to measure guinea pig bone
131 dimensions and map bone local thickness in neonates through to adulthood. Although present
132 literature states that adult weight is achieved at 8-12 months (Hubrecht & Kirkwood 2010), bone
133 growth and local thickness has not been elucidated despite its importance to guinea pig
134 husbandry and clinical care. It is important to highlight that this paper investigates female guinea
135 pigs for a number of reasons. Differences in bone development have been attributed to sex and
136 neuter status (May et al. 1991; Perry et al. 2014; Root et al. 1997) but the additional strain of
137 lactation and pregnancy in the female may further decrease calcium content of bones (Horwits &
138 Smith 1990) and may therefore increase the potential of bone damage in this sex. In the pet and
139 meat industry a greater number of female guinea pigs are present. For example an increased
140 number of females are observed in the meat industry, with around 1 male to every 12 females
141 generally accepted as the norm (Koeslag 1989; Nuwanyakpa et al. 1997). Guinea pigs are herd
142 animals in the wild and live in family units with a dominate male, but males that are strangers
143 will frequently fight, therefore they are more difficult to house together as pets (Donnelly 2010),
144 resulting in pet owners being more likely to choose a male/female or female/female pair, or a
145 group of females. It should also be highlighted that males may not show the same growth rates or
146 bone thickness, however the full weight of 900-1200g for males as opposed to 700-1100 g for the
147 females, is still achieved at 8-12 months (Behrend 2008; Hubrecht & Kirkwood 2010; Meredith
148 & Redrobe 2010). Three methods of bone dimension measurement were utilised - a traditional
149 manual calliper method, a manual measurement of microCT images and an automated microCT
150 analysis. A further aim was to assess the location and number of nutrient foramina and the course
151 of the nutrient canal in every bone, and report on the abundance of both the supratrochlear
152 foramen and the supracondylar foramen in the humerus.

Materials and Methods

Sample collection

Naturally deceased, entire female guinea pigs with known medical and husbandry backgrounds were collected under ethical permissions obtained from The University of Nottingham in accordance with the British Home Office laws and the Veterinary Surgeons Act. All animals were fed on standard, commercially available guinea pig food *ad. lib*. Bones were extracted using manual dissection and grouped according to age - 0-1 month (<1m; n=5), 1-3 months (<3m; n=4), 3-6 months (<6m; n=4), 6 months-1 year (<1yr; n=5) and 1-4 years (<4yr; n=5). The right and left scapula, humerus and femur from each guinea pig were analysed.

MicroCT and bone analysis

Three types of bone measurement were carried out 1) manual bone measurementsas described in Figure 1 using World Precision (UK) digital callipers calibrated to three decimal places. 2) Bone measurements as described in Figure 1 using micro CT images and software. 3) Automated microCT measurements to find the maximal height, depth, width, surface area and volume. Measurements 1 and 2 were compared to ensure no discrepancy between manual and automated methods,

Prior to scanning, individual bones were carefully wrapped in thin sheets of x-ray transparent polyethylene packing foam and placed in 40mm diameter x 50mm height plastic specimen jars. Depending on the size of the bones, each jar could accommodate up to 12 individual bones. Each jar was scanned using a GE Phoenix Nanotom S, X-ray microCT system (GE Sensing and Inspection Technologies GmbH, Wunstorf, Germany). The scan consisted of the collection of 1200 angular projection images in 'Fast' mode at an electron acceleration energy of 110kV and 160µA current. The resulting spatial resolution of the scan to fit the entire pot in the field of view was 24.24µm. Scans were performed in approximately 30 minutes. Following tomographic reconstruction using Datos rec v1.5 (GE Sensing and Inspection Technologies GmbH, Wunstorf, Germany), individual bones were virtually extracted (segmented) from the 3D volumetric data based on their higher x-ray attenuation from the low density packing materials using a combination of object calibration and region growing tools in VGStudioMax V2.2. software (Volume Graphics GmbH, Heidelberg, Germany). The bone measurements (see Fig 1), volume and surface area of the extracted volumes was measured automatically using the isosurface calibration values in the software. Bone maximal length and width was manually measured using

184   the calliper tool in VGStudioMax XY image stack data for each bone was exported in 8 bit
185   grayscale tiff image format. Bone local thickness was measured using the BoneJ (Doube et al.
186   2010) plugin for the open source image quantification and analysis software ImageJ 1.44
187   (Schneider et al. 2012). Compact bone local thickness heat map images were visualised in
188   VGStudioMax. Bone dimensions, volumes and densities were analysed using ANOVA using
189   SPSS (V16; f value 0.778, alpha 0.05, power 80%) statistical software, P<0.05 was considered as
190   a significant difference.

191   Nutrient foramina and canals and the humeral supratrochlear and supracondylar foramina were
192   assessed using both sequential scan x-rays and 3D reconstructions. Nutrient foramina were
193   classified as a cavity that fully breached the entire bone wall, the location of each foramen was
194   recoded alongside the course of the nutrient canal through the bone. Each humerus was also
195   assessed for the presence of both a supratrochlear foramen and a supracondylar foramen.

Results and Discussion

Bone development

A previous study compared CT scanning measurements to standard scientific callipers or assessing the skull base and the craniomaxillofacial dimensions in five humans (Citardi et al. 2001). CT measurements were found to be more accurate and had a better representation of bone anatomy, however there was little significant difference in results, with P values ranging from 0.06 to 1.0 (Citardi et al. 2001). In order to ensure that a similar situation was observed in the smaller guinea pig bone, both microCT and manual measurements were carried out on the scapula, humerus and femur. Our data showed non-significant (P>0.05) variations ranging from 96-110% for each measurement, with 60.5% of the data falling within 5% range of the mean. The larger differences, although non-significant, were observed on the femur (smallest bone), specifically the length from the head to the medial condyle of the femur (also one of the smallest measurements). Therefore CT data was used to compare bone growth data (Fig 2). In addition to data being presented in Figures 2-9, raw data is supplied in Tables S1-S5.

Significant differences (P<0.05 to P<0.0001) in scapula measurements were observed between all ages, for all four measurements (as described in Fig 1, values given in Fig 2A and Table S1) except between 1 year and 4 year old guinea pigs in the width from cranial to caudal angle and width of the elbow joint (Fig 2A, Tables S1 and S2, measurement 2, P>0.05). The maximal width and length were significantly increased at 4 years old in comparison to 1 year old (Fig 3A, P<0.05) as were the scapula volume and surface area (Figs 3B and C, P<0.004 and P<0.0001 respectively).

Significant differences in humeri measurements (as described in Fig 1) were observed between all ages in all measurements (measurements shown in Fig 2B and Tables S1 and S3, P<0.008 to P<0.0001) with the exception of width between the head and greater tubercle between 1 year and 4 years and the width of the elbow joint between 3 and 6 months. Overall however, the maximal width, depth, length, volume and surface areas did not significantly increase between 1 and 4 years old (Fig 4, P>0.05), therefore differing growth rates were observed in comparison to the scapula.

The femur measurements showed significant differences in measurements described in Fig 1 between the different age groups (Figs 2C and 5 and Tables S1 and S4, P<0.018 to P0.0001).

226   Although the length from the head to the medial condyle and maximal width increased from 1

227   year to 4 years old, the maximal length and depth did not (Figs 2C and 5A). In contrast to the

228   humerus though, the volume and surface area of the femur were significantly increased from 1

229   year to 4 years (Figs 5B and C, Table S4, P<0.004 and P<0.0001 respectively).

230

231   Mean scapula local thickness (Figs 6 and 7 and Table S5) significantly increased until 6 months

232   old (P<0.003), but appeared to stabilise thereafter before a slight decrease was observed at 4

233   years old, whereas the maximum scapula local thickness in younger guinea pigs was significantly

234   lower in animals aged 6 months in comparison to 1 year/4 year old bones (Fig 7A, P<0.005 to

235   P<0.0001). Humerus local thickness, both mean and maximum, also significantly increased from

236   1 month to 4 years (P<0.0001 and P<0.002 respectively), but only the mean significantly

237   increased in the latter stages from 6 months to 4 years (Fig 7B, P<0.023). Femur local thickness

238   mean and maximum significantly increased until 1 year (P<0.002) and 6 months (P<0.033)

239   respectively but no differences were observed beyond these ages (Fig 7C).

240   The differential compact bone local thickness can be observed in detail across the developing

241   scapula, humerus and femur in Figure 6 and Table S5. It was interesting to note that higher

242   growth rates were observed in the scapular (flat bone) between years 1 and 4 in comparison to the

243   long bones (Fig 8A, see also Figs 2-5), however the bone local thickness was more likely to

244   increase between these time points in the long bones in comparison with the scapula (Fig 8B, see

245   also Fig 7). Despite these overall increases, it was noted that the older bones had a far greater

246   variation in localised bone thickness, resulting in greater extremes of thickness being observed in

247   comparison to younger bones (Figs 6-8 and Table S5).

248   **Nutrient, supratrochlear and supracondylar foramina**

249   The nutrient foramen and canal are the result of the invading nutrient artery during fetal

250   development (Payton 1934). It was noted in our study that every bone had between 2 and 5

251   nutrient foramina; no bones contained a singular foramen. Within the scapula, the primary

252   foramen is commonly located in most species at the lateral aspect of the infraspinous fossa,

253   whilst secondary foramina (if present) can be located on either the infrasinous fossa or the

254   subscapular fossae (Scheuer et al. 2000). This was also observed in our study, but secondary

255   foramina were also located on the scapula spine and acromion. Within the guinea pig long bones

256   (femur and humerus), the primary nutrient foramina were located in the proximal or distal third

257 of the diaphysis. Further foramina (up to four more per bone) were located: above the trochlear

258 foramen and on the greater tubercle (humerus), and on the head and the shaft of the head (femur).

259 This positioning is similar to most animals where within long bones, the primary nutrient

260 foramen is often located in either the proximal or distal third of the diaphysis, with the canal

261 running obliquely into the medulla (Payton 1934). Previous research in the pig found that in the

262 humerus, the foramen is located in the distal third of the bone and the canal directed proximally,

263 while in the femur the foramen is located in the proximal third and the canal runs distally (Payton

264 1934). In relation to the multiple foramina (Fig 9) observed in the guinea pig bones, it has been

265 reported that each bone generally has a single nutrient foramen, however studies on the canine

266 femur found that only 6.2% of cases contained a single foramen, while more frequently, two or

267 more foramina existed in a single bone (Ahn 2013). Our serial x-ray data showed that every

268 nutrient canal, regardless of bone, position or age, travelled in an oblique direction in the female

269 guinea pig. The oblique direction observed concurs with, and supports, previous studies in both

270 the guinea pig and most other species (De Bruyn et al. 1970; Payton 1934).

The lower end of the humerus has two large fossae, the olecranon fossa and the coronoid fossa,

separated by a thin bony plate that rarely bears an opening known as supratrochlear foramen

(STF). A STF was observed in all of the female guinea pig humeri (n=46), left and right

regardless of age (Fig 9). The SFT is formed when the olecranon fossa at the caudal aspect of the

distal humerus is so deep that is meets the radial fossa on the bone's cranial aspect. The SFT is

closed by a membrane of connective tissue and no major blood vessels of nerves pass through the

foramen. The presence of the SFT within mammals is very variable and is linked to the range of

mobility in the elbow joint. Most hystricognath rodents have a STF, which is likely to facilitate

full extension of the elbow joint in terrestrial locomotion, while in arboreal species the olecranon

fossa is more often shallow and a SFT is not formed (Candela & Picasso 2008). In humans there

is some variability in the presence of an SFT. A study of the humerus in North Indians found that

the presence of the STF varied within the sampled population from not being present at all, to

present bilaterally or only in one femur (Mahajan 2011). 26% of the humeri had a STF, with a

higher frequency in females and in the left humerus (Mahajan 2011). It has been hypothesised

that the presence of a STF may produce stress, altering the fracture lines and possibly increasing

supracondylar fracture rates, even in low-energy trauma (Sahajpal & Pichora 2006). This could

be of clinical concern in guinea pigs as 58% of all human paediatric elbow fractures are in the

supracondylar area of the humerus (Houshian et al. 2001).

271   It was also noted that the supracondylar foramen was absent in all 46 guinea pig humeri. The

272   supracondylar or entepicondylar foramen is a foramen proximal to the medial epicondyle of the

273   humerus. The median nerve and brachial artery pass through this foramen. The supracondylar

274   foramen is an ancestral structure in mammals and has been lost independently in several

275   mammalian clades during mammalian evolution (Polly 2007). The foramen is absent in the

276   guinea pig, as well as in other hystricognath rodents (Candela & Picasso 2008).

Conclusions

277   **Conclusions**

278   Understanding normal bone growth and development in the guinea pig is essential, especially in

279   relation to movement and homeostasis as a store of calcium and phosphorus. Guinea pigs suffer

280   from a number of bone disorders including metabolic bone disease – conditions that develop

281   following prolonged calcium or vitamin D deficiency, or an improper ratio of calcium to

282   phosphorus in the diet (Terril & Clemons 1997). Imbalances have resulted in reports of reduced

283   growth rate, pathological changes of the animal's skeleton and osteodystrophia fibrosa resulting

284   from nutritional secondary hyperparathyroidism (Rapsch Dahinden et al. 2009; Schwarz et al.

285   2001). Bone disorders can also manifest from hypovitaminosis C, a dietary insufficiency of

286   vitamin C for just 2 to 3 weeks can result in lameness or pain due to intra-articular haemorrhage,

287   anorexia, weight loss and general unthriftiness, progressing to death if untreated (Gad 2013;

288   Richardson 2003; Terril & Clemons 1997) and if combined with vitamin E deficiency the time

289   can drop to just a few days (Hill et al. 2003). Disorders such as osteoporosis, common in this

290   species (Bendele et al. 1989), accelerate bone composition and modelling changes and decrease

291   the bone's density significantly, however this can also be attributed to normal age-related changes

292   (Bilezikian et al. 2008). In order to compare and understand disease states, normal bone growth,

293   development and morphology must be categorised and our study has added to the data available

294   on normal bones throughout development.

295   Differences in bone development have also been attributed to sex and neuter status (May et al.

296   1991; Perry et al. 2014; Root et al. 1997), therefore it is important to highlight that the male

297   guinea pig rates of growth and bone development characteristics may differ in relation to our

298   study, as may neutered females. In addition to this, further variation may be seen within the

299   female as lactation and pregnancy are known to alter calcium content of bones (Horwits & Smith

300   1990).

301   Over the years a number of techniques have been used to investigate bone and unravel the

302   complex biology of disorders, for example clinical CT, magnetic resonance and histology.

303   MicroCT holds some benefits such as higher resolution or speed of sample processing in

304   comparison with other techniques but with the caveat that it can only be used in non-living

305   specimens (due to significantly increased x-ray radiation dose required for microCT in relation to

306   clinical CT scanning).

307 Our study is the first to show the growth rates and local bone thicknesses for the scapula,

308 humerus and femur in the developing guinea pig. Despite sexual maturity occurring at around 6-

309 10 weeks, and full animal weight being achieved at 8-12 months, the scapula, humerus and femur

310 continue to grow, and the local bone thicknesses alter beyond 1 year. This paper is also the first

311 to show the absence of the supracondylar foramen and the unusual presence of the supratrochlear

312 foramen in the humerus of this species, which may in turn affect fracture rates and locations

313 within the humerus. Understanding guinea pig bone development and anatomy can help inform

314 clinical and husbandry practice, especially in relation to bone thickness and fractures.

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Figure 1

Figure 1: Gross anatomical photographs indicating measurements calculated.

A) Scapula; 1- length of the bone; 2- length of the spine; 3- width from cranial to caudal angle; 4- width of the shoulder joint. B) Humerus; 1-length from the head to the elbow; 2- width from the head to the greater tubercle; 3- width of the elbow joint and C) Femur; 1- overall length from the head to the medial condyle.

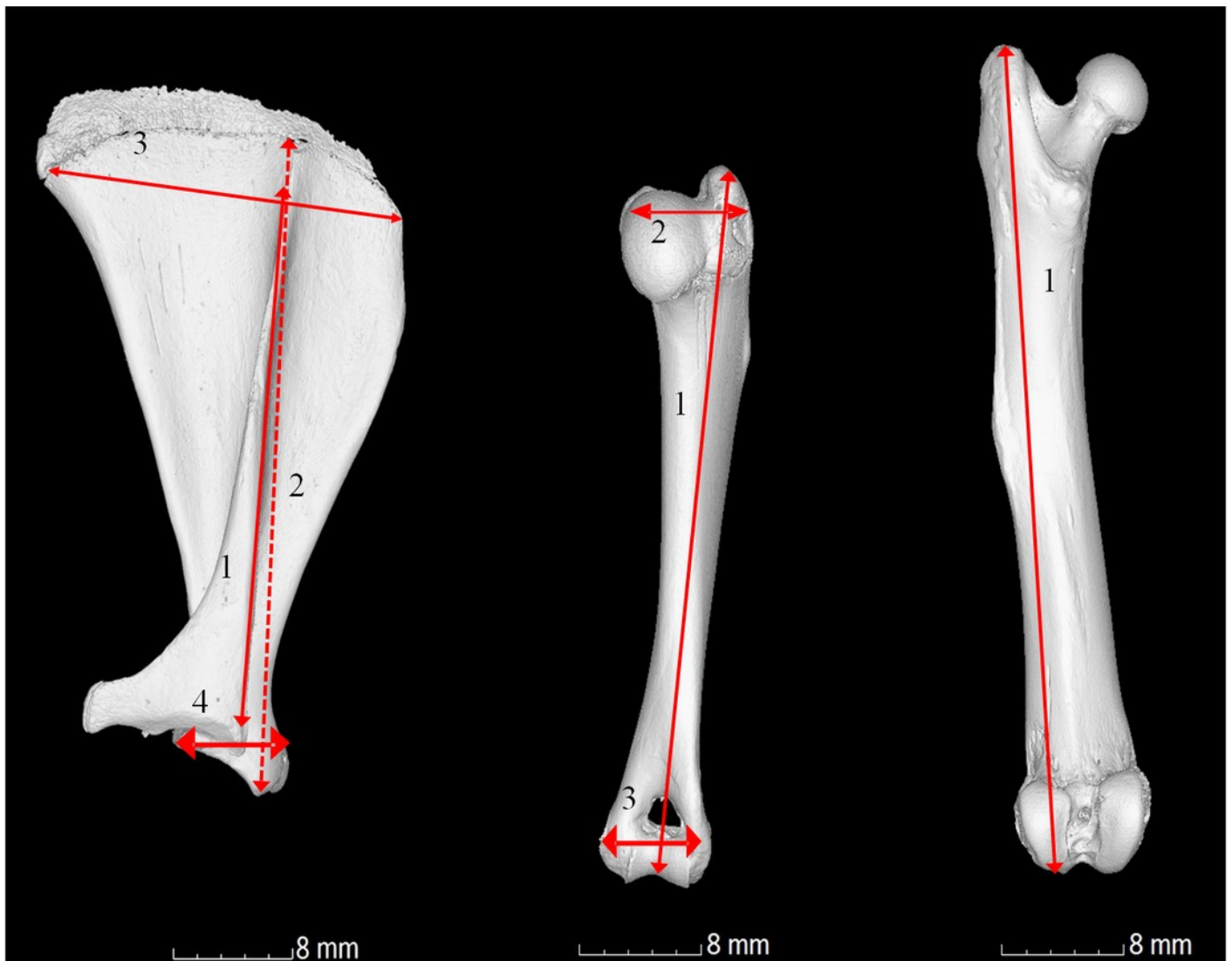



Figure 2

Figure 2 Manual measurements aided by micro-CT.

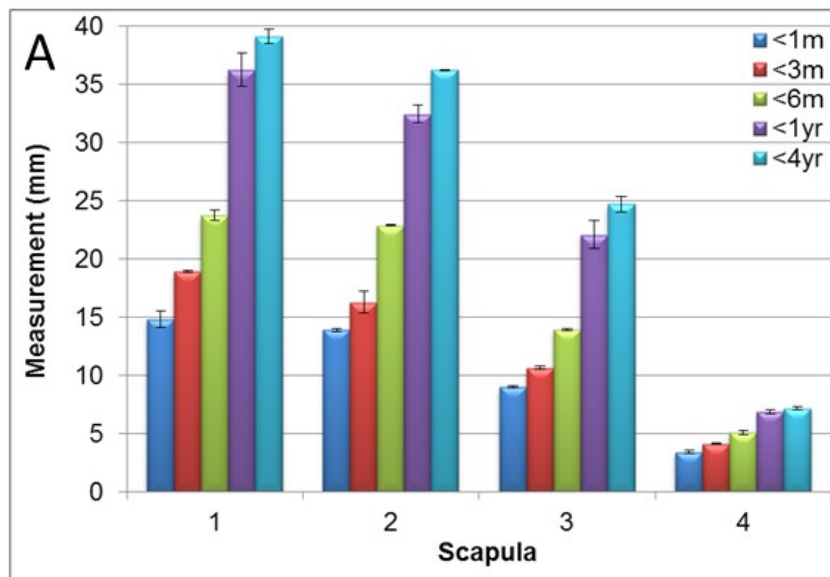
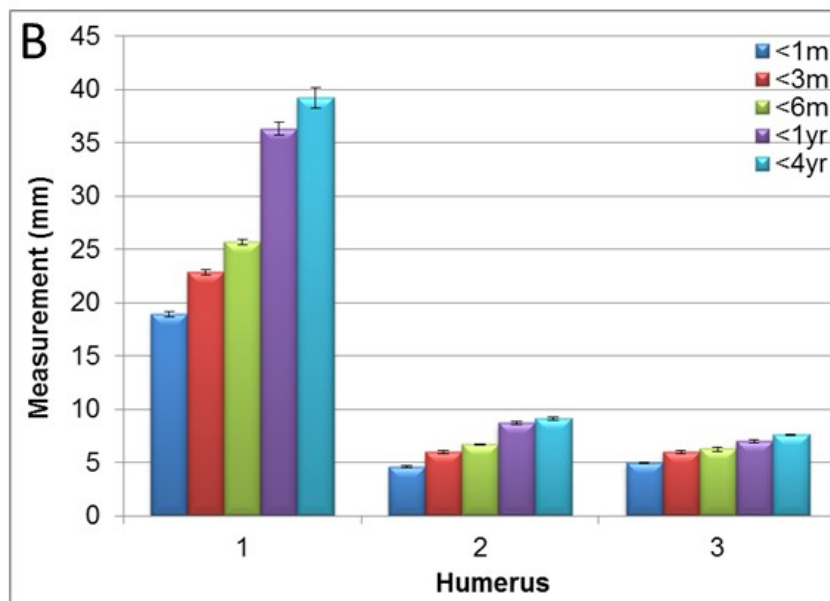
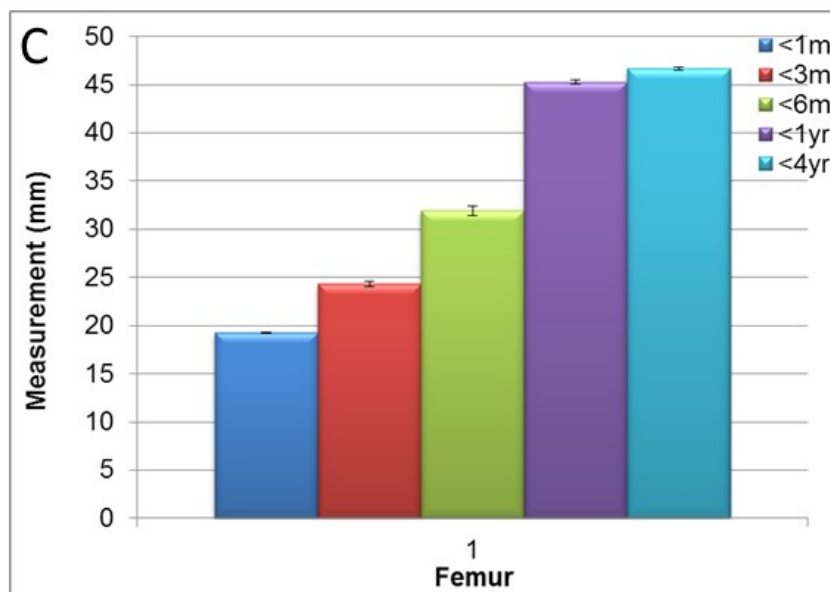
Measurements of guinea pig bones aged 0-1 month (<1m), 1-3 months (<3m), 3-6 months (<6m), 6 months-1 year (<1yr) and 1-4 years (<4yr). A) Scapula, B) humerus and C) femur with associated ANOVA with post-hoc P value tables. NS=not significant. Mean ± standard error of the mean error bars.

Scap 1 (mm)	<3m	<6m	<1yr	<4yr
<1m	0.002	0.0001	0.0001	0.0001
<3m	X	0.001	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	NS
Scap 2 (mm)	<3m	<6m	<1yr	<4yr
<1m	0.049	0.0001	0.0001	0.0001
<3m	X	0.0001	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	0.001
Scap 3 (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	0.0001	0.0001	0.0001
<3m	X	0.005	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	0.012
Scap 4 (mm)	<3m	<6m	<1yr	<4yr
<1m	0.011	0.0001	0.0001	0.0001
<3m	X	0.001	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	NS

Hum 1 (mm)	<3m	<6m	<1yr	<4yr
<1m	0.0001	0.0001	0.0001	0.0001
<3m	X	0.0001	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	0.001
Hum 2 (mm)	<3m	<6m	<1yr	<4yr
<1m	0.0001	0.0001	0.0001	0.0001
<3m	X	0.0001	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	NS
Hum 3 (mm)	<3m	<6m	<1yr	<4yr
<1m	0.0001	0.0001	0.0001	0.0001
<3m	X	NS	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	0.008

Femur (mm)	<3m	<6m	<1yr	<4yr
<1m	0.0001	0.0001	0.0001	0.0001
<3m	X	0.0001	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	0.018

Figure 3

Figure 3 Micro-CT scapula measurements.

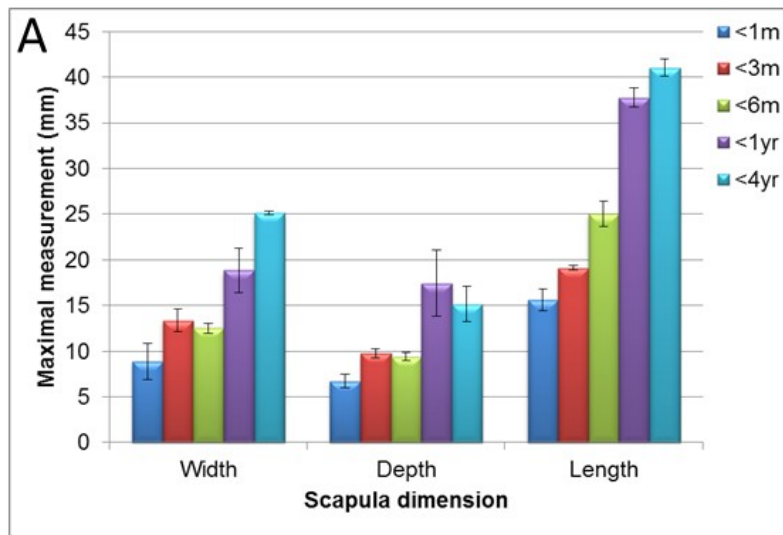
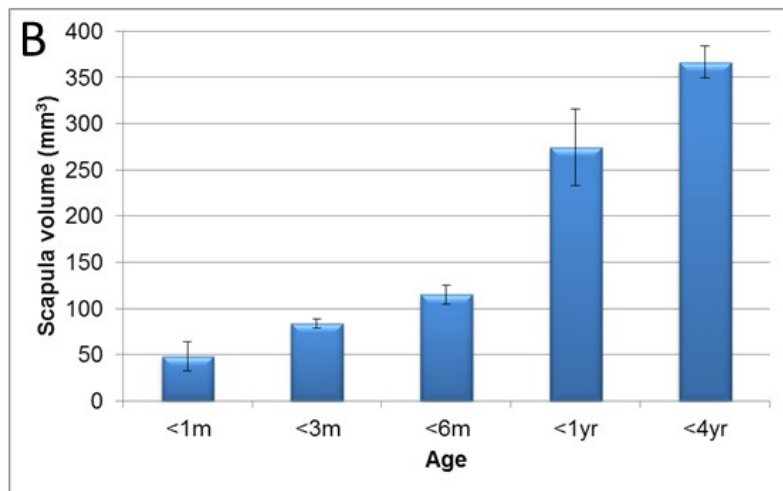
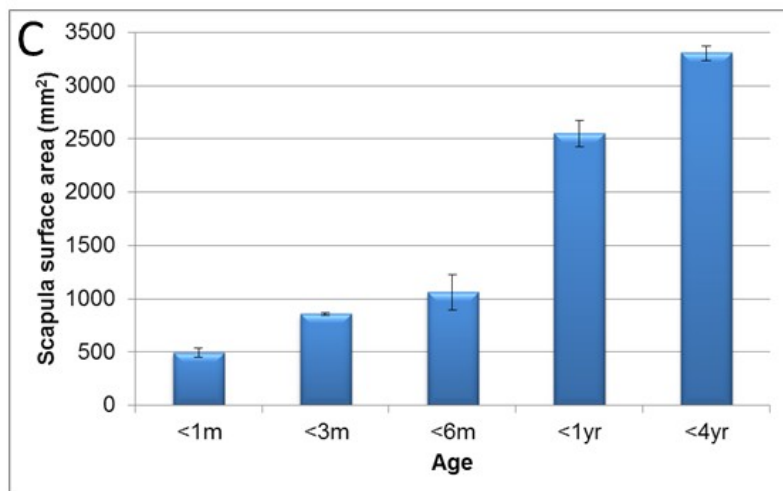
Measurements of guinea pig scapula aged 0-1 month (<1m), 1-3 months (<3m), 3-6 months (<6m), 6 months-1 year (<1yr) and 1-4 years (<4yr). A) bone width, depth and length, B) surface area and C) volume with associated ANOVA with post-hoc P value tables. NS=not significant. Mean ± standard error of the mean error bars. SA=surface area.

Width (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	NS	0.0001	0.0001
<3m	X	NS	0.047	0.001
<6m		X	0.005	0.0001
<1yr			X	0.011
Depth (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	NS	0.0001	0.006
<3m	X	NS	0.033	NS
<6m		X	0.008	NS
<1yr			X	NS
Length (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	0.0001	0.0001	0.0001
<3m	X	0.002	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	0.038

Volume (mm^3)	<3m	<6m	<1yr	<4yr
<1m	NS	0.03	0.0001	0.0001
<3m	X	NS	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	0.004

SA (mm^2)	<3m	<6m	<1yr	<4yr
<1m	0.029	0.0001	0.0001	0.0001
<3m	X	NS	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	0.0001

Figure 4

Figure 4 Micro-CT humerus measurements.

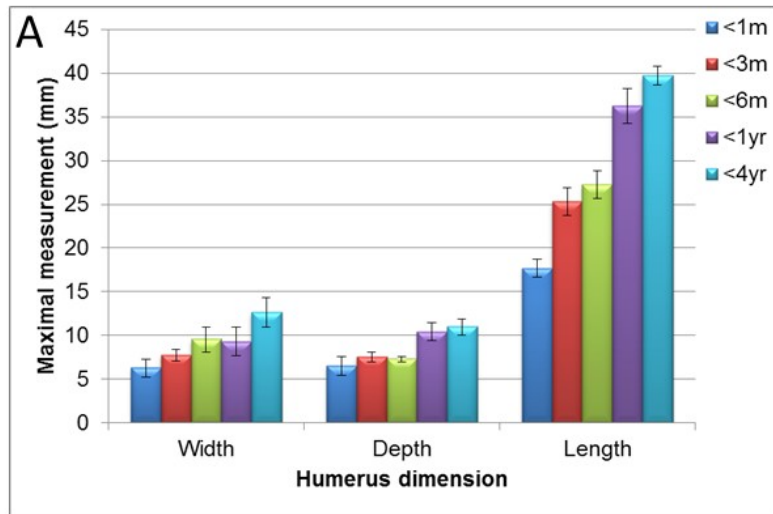
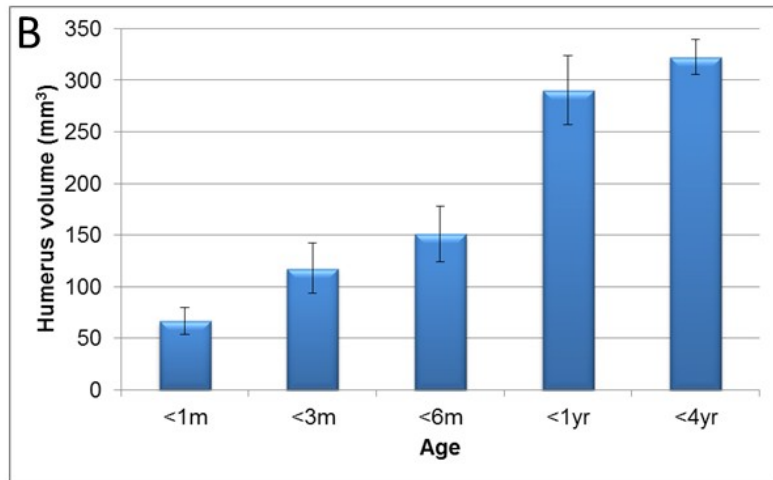
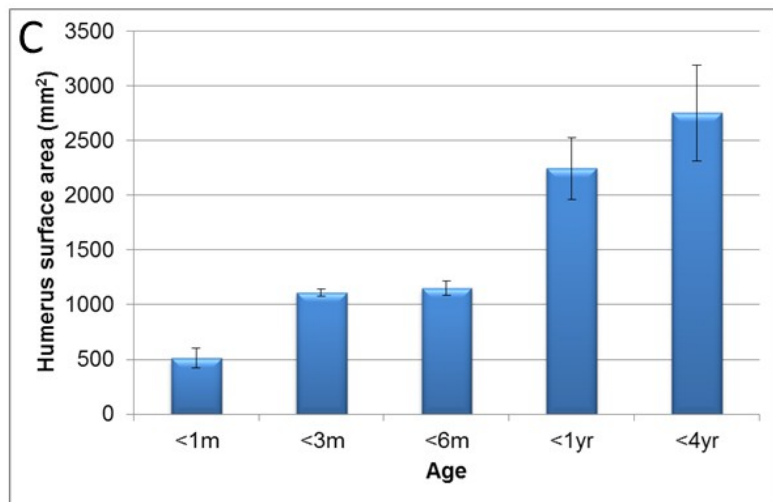
Measurements of guinea pig humerus aged 0-1 month (<1m), 1-3 months (<3m), 3-6 months (<6m), 6 months-1 year (<1yr) and 1-4 years (<4yr). A) bone width, depth and length, B) surface area and C) volume with associated ANOVA with post-hoc P value tables. NS=not significant. Mean ± standard error of the mean error bars. SA=surface area.

Width (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	0.043	0.05	0.0001
<3m	X	NS	NS	0.015
<6m		X	NS	NS
<1yr			X	NS
Depth (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	NS	0.005	0.003
<3m	X	NS	NS	0.036
<6m		X	0.031	0.019
<1yr			X	NS
Length (mm)	<3m	<6m	<1yr	<4yr
<1m	0.0001	0.0001	0.0001	0.0001
<3m	X	NS	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	NS

Volume (mm^3)	<3m	<6m	<1yr	<4yr
<1m	NS	0.005	0.0001	0.0001
<3m	X	NS	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	NS

SA (mm^2)	<3m	<6m	<1yr	<4yr
<1m	0.015	0.005	0.0001	0.0001
<3m	X	NS	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	NS

Figure 5

Figure 5 Micro-CT femur measurements.

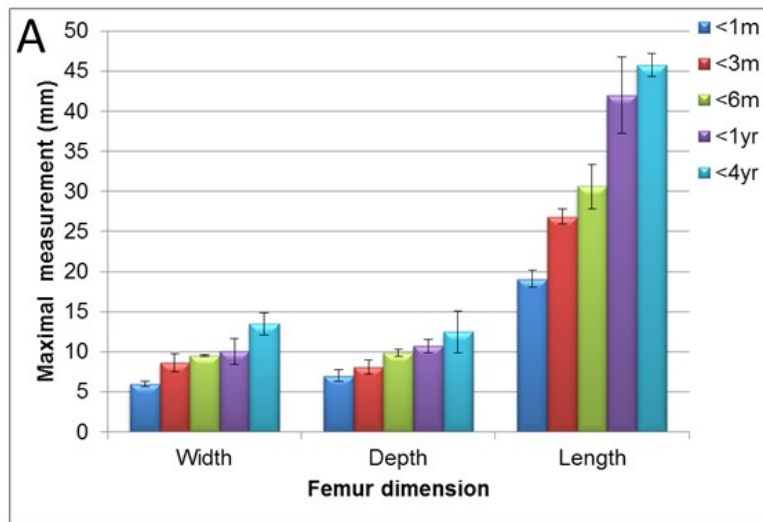
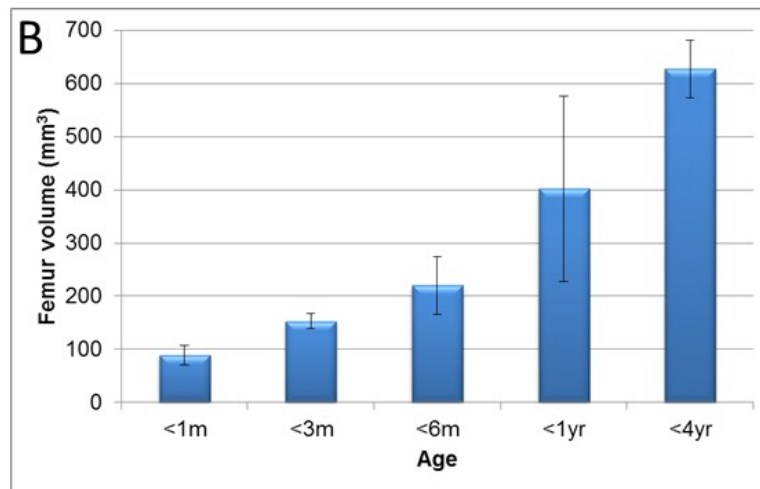
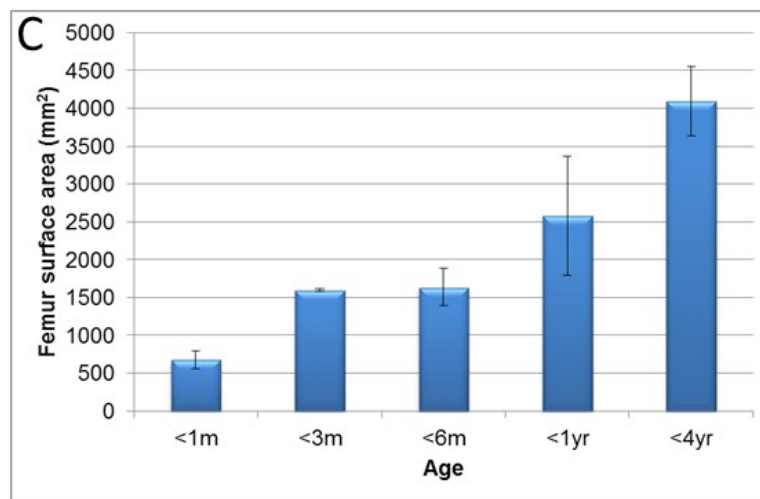
Measurements of guinea pig femur aged 0-1 month (<1m), 1-3 months (<3m), 3-6 months (<6m), 6 months-1 year (<1yr) and 1-4 years (<4yr). A) bone width, depth and length, B) surface area and C) volume with associated ANOVA with post-hoc P value tables. NS=not significant. Mean ± standard error of the mean error bars. SA=surface area.

Width (mm)	<3m	<6m	<1yr	<4yr
<1m	0.014	0.001	0.001	0.0001
<3m	X	NS	NS	0.0001
<6m		X	NS	0.0001
<1yr			X	0.002
Depth (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	0.024	0.012	0.0001
<3m	X	NS	NS	0.004
<6m		X	NS	0.046
<1yr			X	NS
Length (mm)	<3m	<6m	<1yr	<4yr
<1m	0.001	0.0001	0.0001	0.0001
<3m	X	NS	0.0001	0.0001
<6m		X	0.0001	0.0001
<1yr			X	NS

Volume (mm^3)	<3m	<6m	<1yr	<4yr
<1m	NS	0.034	0.0001	0.0001
<3m	X	NS	0.001	0.0001
<6m		X	0.006	0.0001
<1yr			X	0.004

SA (mm^2)	<3m	<6m	<1yr	<4yr
<1m	0.005	0.004	0.0001	0.0001
<3m	X	NS	0.012	0.0001
<6m		X	0.007	0.0001
<1yr			X	0.0001

Figure 6

Figure 6 Micro-CT bone local thickness heat mapping reconstructions.

Representative heat mapping reconstructions of guinea pig bone localised thickness aged 0-1 month (<1m), 3-6 months (<6m) and 1-4 years (<4yr). From left to right: Scapula – anterior and posterior, humerus – anterior and posterior and femur – anterior and posterior. Scale bars for each age represent 8mm.

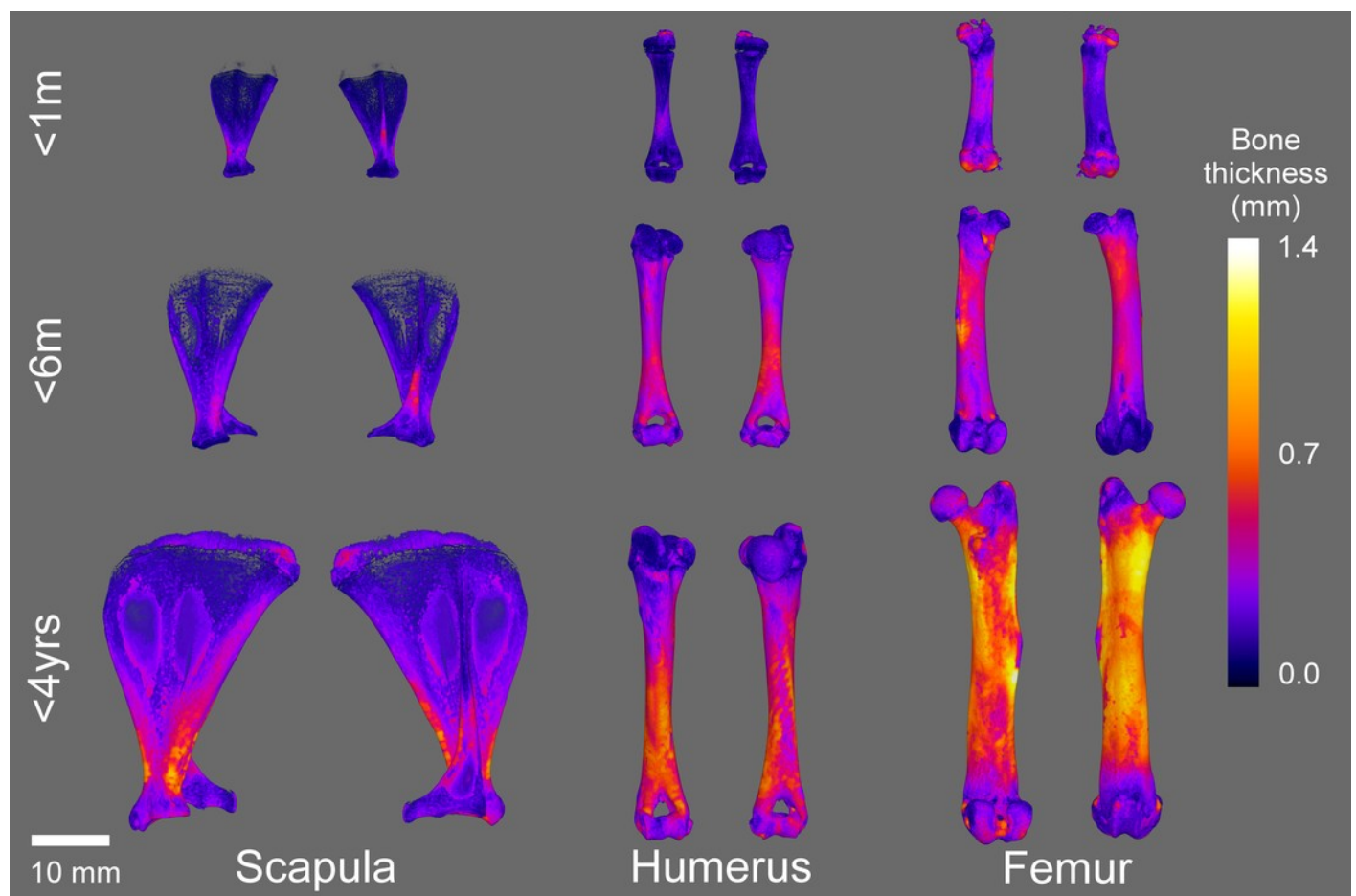
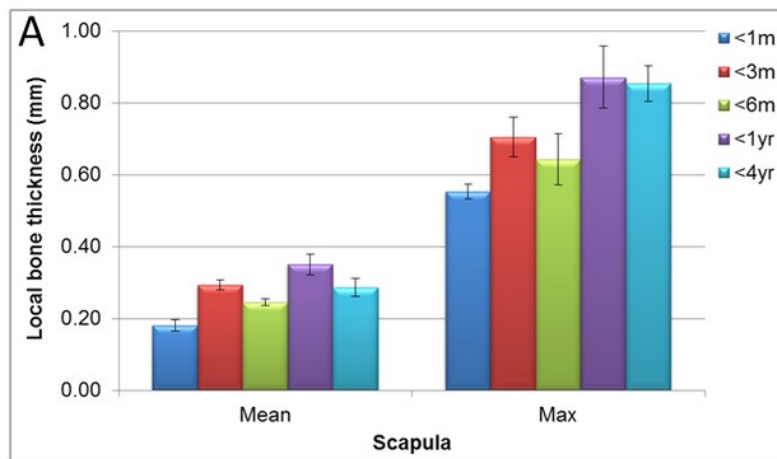
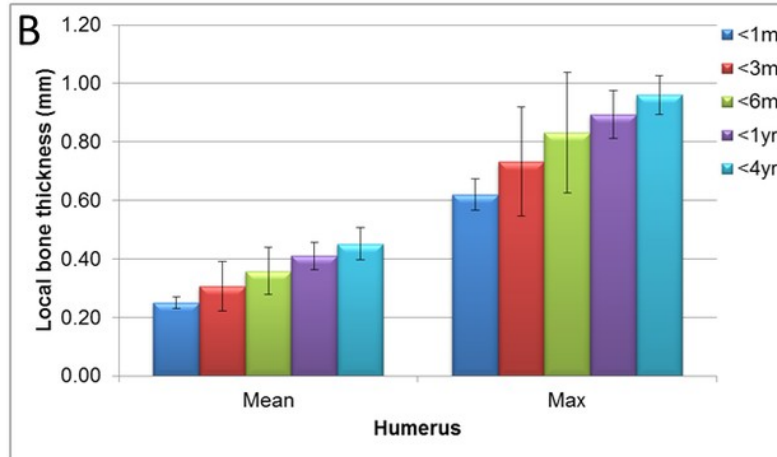
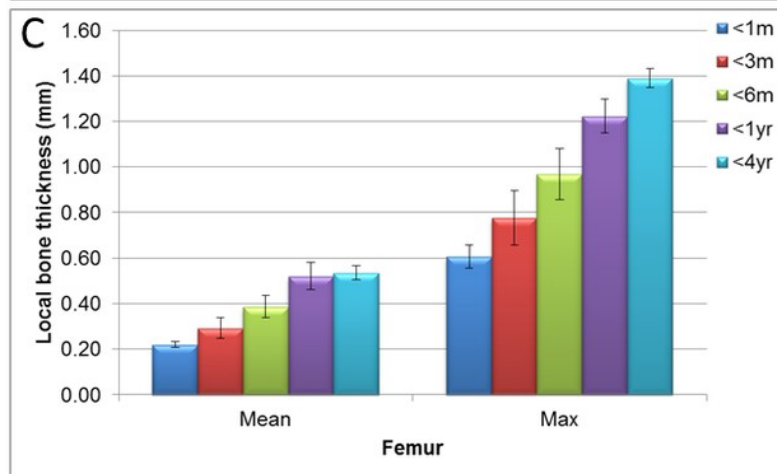



Figure 7

Figure 7 Micro-CT bone local thickness measurements.

Measurements of guinea pig bone local thickness aged 0-1 month (<1m), 1-3 months (<3m), 3-6 months (<6m), 6 months-1 year (<1yr) and 1-4 years (<4yr). A) scapula, B) humerus and C) femur with associated ANOVA with post-hoc P value tables. NS=not significant. Mean ± standard error of the mean error bars.

Mean (mm)	<3m	<6m	<1yr	<4yr
<1m	0.003	0.028	0.0001	0.0001
<3m	X	NS	NS	NS
<6m		X	0.003	NS
<1yr			X	0.022

Max (mm)	<3m	<6m	<1yr	<4yr
<1m	0.042	NS	0.0001	0.0001
<3m	X	NS	0.0001	0.0001
<6m		X	0.005	0.005
<1yr			X	NS

Mean (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	NS	0.002	0.0001
<3m	X	NS	NS	0.014
<6m		X	NS	0.023
<1yr			X	NS

Max (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	NS	0.011	0.002
<3m	X	NS	NS	NS
<6m		X	NS	NS
<1yr			X	NS

Mean (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	0.0001	0.0001	0.0001
<3m	X	0.015	0.0001	0.0001
<6m		X	0.02	0.012
<1yr			X	NS

Max (mm)	<3m	<6m	<1yr	<4yr
<1m	NS	0.0001	0.0001	0.0001
<3m	X	0.033	0.0001	0.0001
<6m		X	NS	0.002
<1yr			X	NS

Figure 8

Figure 8 Bone growth and localised thickness throughout development.

Percentage bone growth (A) and local thickness (B) of 0-1 month (<1m), 1-3 months (<3m), 3-6 months (<6m), 6 months-1 year (<1yr) old guinea pigs in comparison to 1-4 year olds.

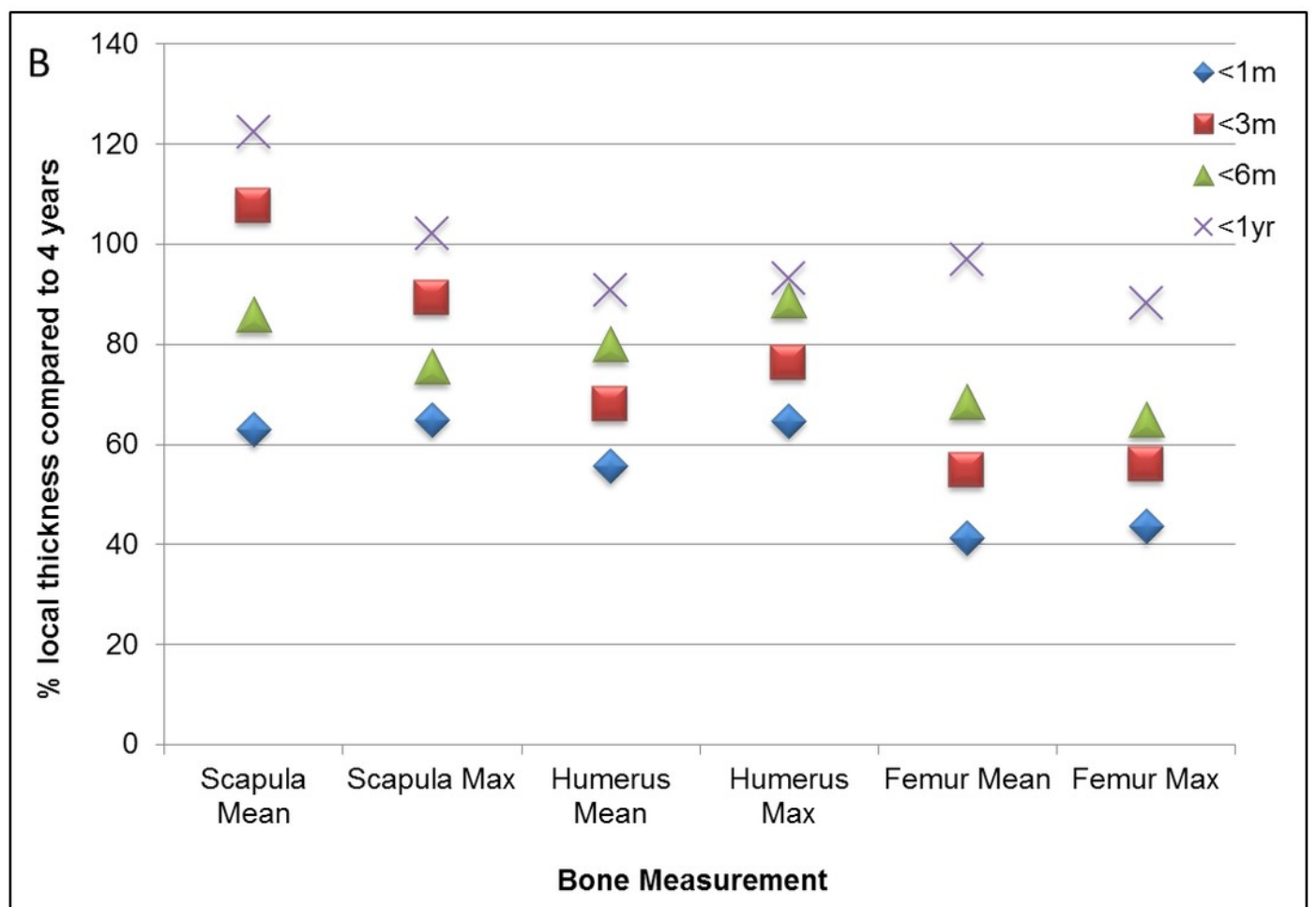
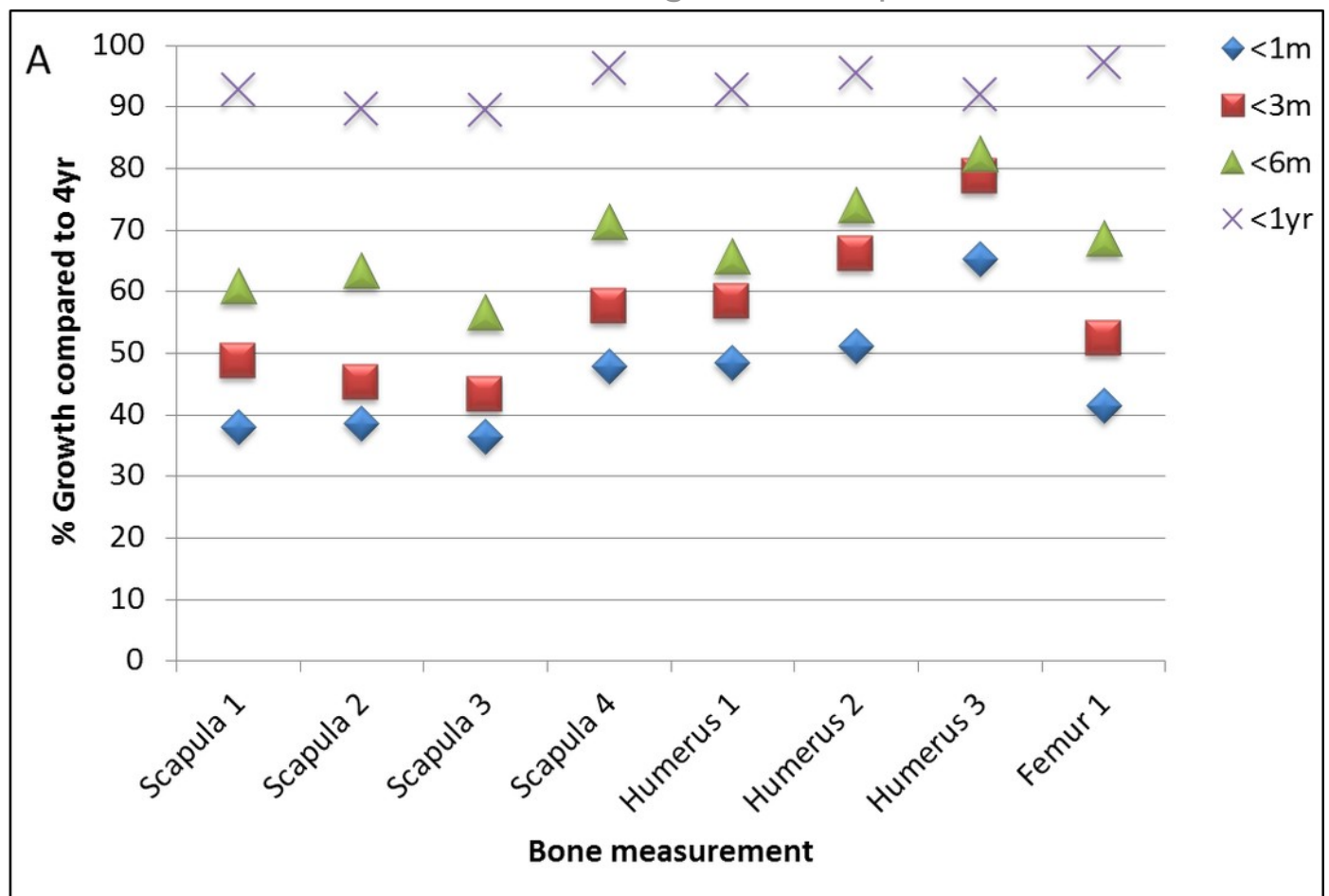



Figure 9

Figure 9 Gross anatomical features of the humerus.

Humerus anterior (top row) and posterior (lower row) views showing examples of the supratrochlear foramen (as indicated by closed arrowhead) at 0-1 month (<1m), 1-3 months (<3m), 3-6 months (<6m), 6 months-1 year (<1yr) and 1 year-4 years (<4yr). Examples of nutrient foramina in a 4 year old humerus are indicated (open arrowhead). Scale bars represent 8mm.

<1m **<3m** **<6m** **<1yr** **<4yr** **Nutrient foramina**