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Trabecular and subchondral bone development of the talus and distal tibia from foal to adult in the warmblood horse

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Summary

Horses are precocial animals and able to stand and walk within hours after birth. To cope with associated loading, intrauterine bone development has shown to be anticipative. This study provides further insight into the post-natal development of structurally important features of trabecular and subchondral bone of the talus and sagittal ridge of the tibia of warm-blooded horses. In all areas studied, the average bone volume fraction showed a gradual increase over time, which was the result of a significant increase in trabecular thickness, without significant changes in the degree of anisotropy. Similar to the mineralised part of the bone, collagen content, measured as average retardation using polarised light microscopy, increased significantly, but the degree of anisotropy of the collagen type I network did not. At birth, the subchondral bone layer had a more trabecular aspect, gradually changing to an even surface with only a few vascular canals at an age of 2 months. Presented results indicate the necessity for a stronger structure, but not for a different structural design after birth, providing further evidence for anticipatory bone development in the horse. More knowledge about the strategies used to cope with mechanical loading after birth might be helpful in understanding the developmental bone and joint diseases.

1 | INTRODUCTION

According to Wolff's law on bone adaptation (Wolff, 1892) and the Mechanostat theory (Frost, 2001), bone adapts reactively to loading by decreasing bone mass when loaded below a certain threshold and increasing it in reaction to loading above this threshold, achieving an optimal balance between bone weight and strength. However, precocial animals need to stand and outrun predators within hours after birth. To withstand the forces associated with this, fetal bone development has shown to be anticipative, reflecting future rather than past function, both in calves and in foals (Gorissen, Wolschrijn, Van Vilsteren, Van Rietbergen, & Van Weeren, 2016). This phenomenon, which is possibly based on a genetic blueprint, seems general as it has been described in several other precocial species (Skedros, Hunt,

& Bloebaum, 2004; Skedros, Sorenson, Hunt, & Holyoak, 2007) and even in altricial species (Cunningham & Black, 2009).

After birth, the biomechanical situation changes dramatically and trabecular bone development is strongly influenced by loading, as has been shown in infants (Acquaah, Robson Brown, Ahmed, Jeffery, & Abel, 2015; Gosman & Ketcham, 2009; Ryan & Krovitz, 2006) and puppies (Wolschrijn & Weijs, 2004). Their altricial behaviour after birth is responsible for the initial reduction of trabecular bone volume fraction (BV/TV; fraction that is mineralised (BV) of a given volume of bone (total volume, TV)). As the reduction in the BV/TV, at least in babies, coincides with relatively high levels of parathyroid hormone (David & Anast, 1974; Land & Schoenau, 2008; Rubin, Posillico, Anast, & Brown, 1991), it has been suggested that the relatively high bone volume at birth might serve as a reservoir

for calcium during bone growth (Acquaah et al., 2015). After the onset of locomotion, BV/TV in these species increases again in reaction to the higher loading, making the curve describing BV/TV after birth U-shaped. Additionally, a certain degree of anisotropy develops, because trabeculae aligned with loading will grow in thickness, whereas trabeculae that are not will shrink and ultimately disappear (Tanck, Homminga, van Lenthe, & Huiskes, 2001; Wolschrijn & Weijs, 2004). Given the extreme precocial behaviour of many of the larger ungulates, including equidae, one might expect a different BV/TV curve with less bone loss and most likely an immediate gradual increase in trabecular BV/TV after birth, in line with what is seen in altricial species after the onset of walking, but this has not been reported yet. In the same line of thinking, it can be hypothesised that unlike in altricial species, directional anisotropy (DA) will not change significantly after birth in the foal.

Other features of epiphyseal bone that might behave differently in altricial and precocial species include the density of the subchondral bone layer and characteristics of the collagen type I network. The subchondral bone layer supports the overlying cartilage (Brama, Bank, Tekoppele, & Van Weeren, 2001; Radin & Rose, 1986) and transmits forces to the underlying trabecular bone (Holopainen et al., 2008). Its development is mechanically driven (Skedros et al., 2007; Tanck et al., 2006; Wolschrijn & Weijs, 2004), as solid patches of subchondral bone were shown to appear first on the locations where bone loading is highest (Wolschrijn & Weijs, 2005). Given the extreme precocial behaviour and associated high loading of the skeleton in equids, it is plausible to expect these patches to develop early in foals. Due to the fact that a solid subchondral bone layer limits further growth by endochondral ossification (Carter, 1987; Radin, Schaffler, Gibson, & Tashman, 1995), a delicate balance between loading resistance and growth requirements will have to be maintained.

The collagen type I network comprises about 90% of the non-mineralised matrix of bone (Buckwalter, Glimcher, Cooper, & Recker, 1995) and is of major influence on the strength of the bone. This network is laid down before calcification takes place, and it has been postulated that it plays a leading role in regulating mineralisation (Wang et al., 2012; Wassen et al., 2000). This would mean that the changes in trabecular bone orientation during the early juvenile phase would occur simultaneously with or be preceded by changes in collagen orientation. Indirectly, this assumption is supported by the observations that both in the altricial rabbit (Turunen, Saarakkala, Helminen, Jurvelin, & Isaksson, 2012) and in the precocial foal (Holopainen et al., 2008), the collagen network of the cortical and subchondral bone reaches a mature organisation far before the mineralised part of the bone does.

The aim of this study is to elucidate the post-natal development of structurally important parameters in the subchondral bone of the distal tibia and talus of Dutch warm-blooded foals using micro–computed tomography (micro-CT) imaging and polarised light microscopy (PLM). We hypothesise that (i) the BV/TV curve of the trabecular bone will not show a U-shape, but a gradual increase; (ii) trabecular DA will show only minor changes during growth; (iii) a patched subchondral bone layer, with solid patches on the locations where most bone loading can be expected, is present in young foals; and (iv) there are no or only minor differences in the orientation and degree of anisotropy of the collagen type I network after birth.

2 | MATERIALS AND METHODS

2.1 | Animals

Tarsal joints of Dutch warm-blooded foals and adult horses (*Equus ferus caballus*) that were stillborn or euthanised due to reasons unrelated to the tarsocrural joint or general orthopaedic disorders were obtained with owners' consent from private veterinary practices and the (para)clinical departments of the Faculty of Veterinary Medicine, Utrecht University. Five neonatal foals (0–12 hr old) and four older foals (63, 65, 81 and 145 days old) were included. For comparison, three adult (age range 12–20 years old) warm-blooded animals were also included. The micro-CT data of the 5 neonatal foals have been used as well in a previous study on early skeletal development (Gorissen et al., 2016).

2.2 | Sample preparation

Sample preparation and micro-CT imaging were carried out as described previously (Gorissen et al., 2016). In short, tarsal joints were collected within two hours after death and stored cooled (4°C) if processing could be carried out within 12 hr or frozen (−18°C) when this took longer. After the removal of all soft tissue, the articular surfaces of the distal tibia and talus were macroscopically inspected and photographed. Samples were subsequently fixed in 4% buffered formaldehyde for at least a week. To fit in the micro-CT sample holder (diameter 7.85 cm), distal tibias were cut transversely through the distal metaphysis about 1 cm proximal to the distal physis and tali were cut in the longitudinal direction through the middle of the talar trochlea. If necessary, a small piece of the proximal part of the trochlear ridges of the talus was removed.

2.3 | Micro-CT

Micro-CT imaging was conducted with a μCT 80 scanner (Scanco Medical AG, Brüttisellen, Switzerland), fitted with an aluminium filter to reduce beam-hardening effects. Bone samples were positioned in the sample holder in a consistent manner and secured using synthetic foam. Scanning was carried out in air at an isotropic spatial resolution of 37 μm, a peak voltage of 70 kV and intensity (current) of 114 μA.

Trabecular bone was quantitatively determined in manually drawn volumes of interest (VOIs) only including trabecular bone. Selected VOIs were in line with previous work (Gorissen et al., 2016). The tibial VOI contained the trabecular bone of the dorsal 25% of the sagittal ridge. The lateral talar VOI consisted of the distal part of the lateral talar ridge, and the medial talar VOI contained the middle region of the medial talar ridge. The subchondral bone layer was

analysed in these same areas. As there was no clear subchondral bone layer visible in the neonatal foals, a layer of 40 voxels thickness (40 × 37 μm = 1,480 μm), just under the cartilage, was selected to represent this layer.

Based on the histograms and visual comparison of segmented images at different threshold levels with the original scans (Wolschrijn & Weijs, 2004), a threshold of 145/1,000 of the maximum possible voxel value (246 mgHA/cm^3) was chosen as best fit to analyse the juvenile bone samples. The samples of the adult horses were analysed at 170/1,000, corresponding to 327 mgHA/cm^3.

Quantitative trabecular and subchondral bone parameters were calculated from the segmented images using the Scanco Medical software. Bone volume was calculated by dividing the number of bone voxels by the total number of voxels in the VOI. All structural parameters, including trabecular number (Tb.N.), trabecular thickness (Tb.Th.) and trabecular separation (Tb.Sp.), were obtained using a distance transformation method. The DA was based on the mean intercept length fabric tensor and defined as the largest principal fabric value over the smallest one. Subchondral bone density was calculated by defining the subchondral bone layer as mentioned above. After thresholding, BV/TV and density of the tissue above the threshold were measured.

2.4 | Histology

From each region of interest analysed by micro-CT imaging, a 0.5-cm-thick sample was cut with a handsaw and subsequently decalcified in 10% ethylenediaminetetraacetic acid (EDTA, Amresco LLC Solon, USA). After embedding in paraffin, three 5-μm-thick sections were cut, which were left unstained and covered using Eukitt (Sigma-Aldrich, Darmstadt, Germany). Polarised light microscopy was carried out with an Abrio PLM system (CRi, Inc., Woburn, USA), consisting of a green band-pass filter, a circular polariser and a computer-controlled analyser with two liquid crystal polarisers and a CCD camera mounted on a conventional light microscope (Nikon Diaphot TMD, Nikon, Inc., Shinagawa, Tokyo, Japan).

Four (talus) or three (tibia) pictures (size of one image: 2.59 × 3.52 mm), covering the total width of trabecular bone of the sample, were made of each slide. Two images were produced, one for the calculation of optical retardance and the other one for measuring the angular orientation of birefringent structures (e.g. collagen). As optical retardance depends on the amount of birefringent material and its anisotropy, it can provide an estimate of the collagen content in the tissue (Rieppo et al., 2007). Retardance and orientation images were analysed using custom MATLAB (Matlab r2015b, Natick, USA) scripts. First, the retardance image was used to remove the empty space due to pores from the analysis. Then, the mean

orientation angles were calculated for each image. The randomness of the orientation angles was quantified by calculating the entropy of the orientation values. The entropy was defined as follows:

$$E = -\sum_i P_i \log_2(P_i)$$

where P_i contains the count of each orientation angle within the window. The anisotropy was calculated as follows:

$$A = \frac{1}{E+1}$$

As the minimum of entropy is 0, the range of this anisotropy parameter is between 0 and 1 (with 1 representing only one orientation angle within the analysis window and 0 representing a situation where all orientation angles within the analysis windows are represented evenly).

2.5 | Statistical analyses

All statistical analyses were conducted using SPSS Statistics 22 (IBM Corporation). To analyse the micro-CT and PLM data, a linear mixed-effect model with foal ID added as random intercept was made using a backward approach based on the Akaike's information criterion (AIC). To meet the normality and homoscedasticity assumptions of the model, several parameters needed transformation (Tb.N, density of the trabecular and subchondral bone, average and SD of the retardation data were square-rooted; Tb.Sp and BV/TV of the subchondral bone layer were logarithmically transformed). Unfortunately, it was not possible to make the SD of the orientation and the anisotropy data of the PLM analysis fit to the model assumptions, and therefore, the nonparametric Mann–Whitney U test was used for these data. Statistical significance was set at p ≤ .05, and outcomes were corrected for multiple comparisons using the false discovery rate method of Benjamini and Hochberg (1995).

## 3 | RESULTS

As there were no significant differences between left and right limbs, these data were pooled.

3.1 | Trabecular bone

Trabecular bone parameters for the analysed VOIs are presented in Figure 1. After birth, average BV/TV increased significantly in all three analysed VOIs. Differences observed between the older foals and adult horses were not significant (Figure 1a). Only the difference in Tb.N. between the neonatal foals and adult horses in the medial

FIGURE 1 Mean (±SD) trabecular bone parameters calculated for the different volumes of interest (VOIs, Lat. talus: lateral part of the talus; Med. talus: medial part of the talus; Tibia: sagittal ridge of the tibia) and age groups (NB: newborn foals; OF: older foals; AD: adult horses). *.01 < p < .05; **p < .01. (a) Bone volume fraction (BV/TV). (b) Trabecular number (Tb.N.). (c) Trabecular thickness (Tb.Th.). (d) Trabecular separation (Tb.Sp.). (e) Directional anisotropy (DA). (f) Density
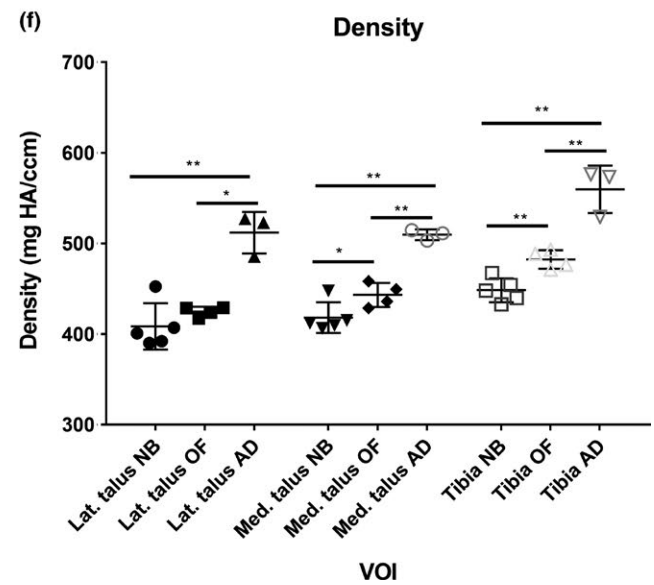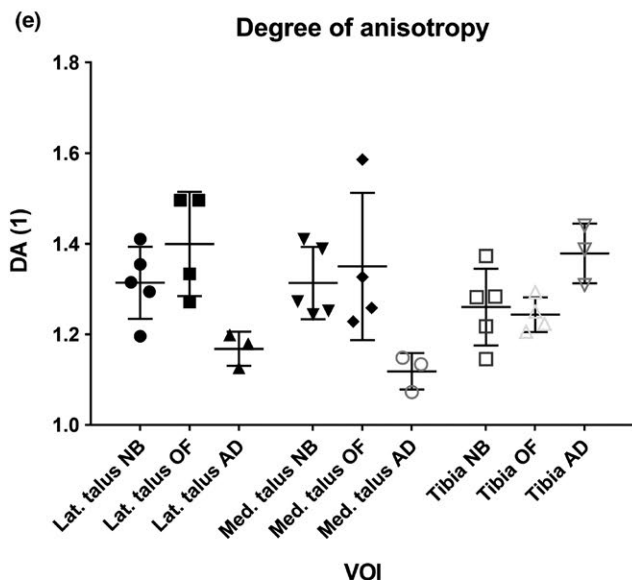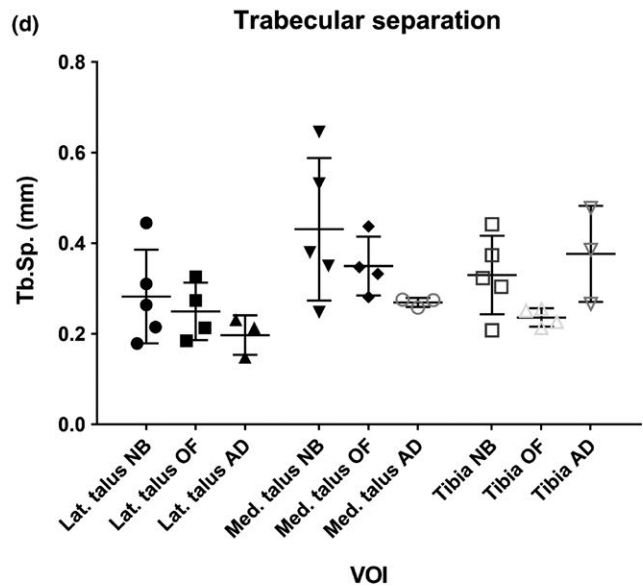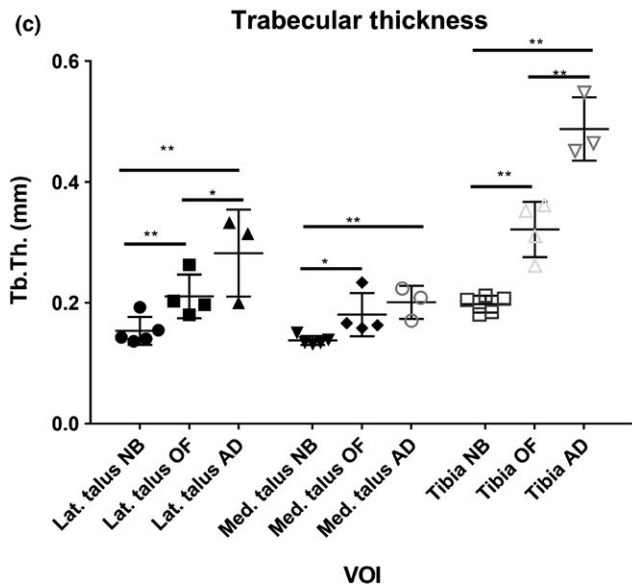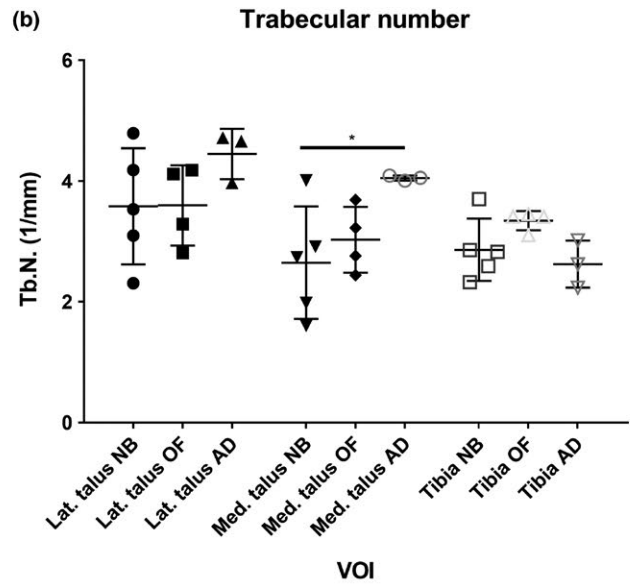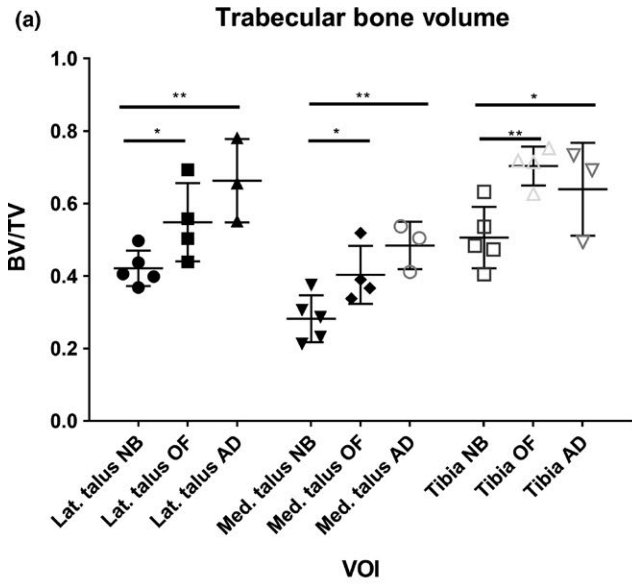
part of the talus was significant (Figure 1b). Compared to the neonatal foals, Tb.Th was significantly higher in all three regions of both the older group of foals and adult horses. In both the tibia and the lateral parts of the talus, trabeculae were significantly thicker in adult horses than in the group of older foals, whereas in the medial part of the talus, this was not the case (Figure 1c). In all VOIs and age groups studied, no significant differences in Tb.Sp. and DA were observed (Figures 1d,e).

Bone density increased gradually with increasing age, except for the lateral talus where newborn and older foals had the same density (Figure 1f).

3.2 | Subchondral bone

The results of the subchondral bone analysis are presented in Figure 2. At birth, no solid subchondral bone layer was present in any of the foals and the average percentage of bone was about 70% for all studied VOIs. In the older group of foals, this volume increased to values of over 80%, which is significant in the lateral talus (Figure 2a). A strong and significant increase in the degree of mineralisation of all regions was observed from newborns to older individuals (Figure 2b). See for illustration Figure 2c, which shows representative examples of the visual aspects of the subchondral bone at birth and later in life.

3.3 | Collagen network

In all VOIs and depths studied, a significant increase in average retardation was observed (Table 1). Also, the variation in the retardation signal significantly increased in the older group of foals. The average orientation of the collagen type I network only changed in the deeper parts of the medial trochlear ridge of the talus and stayed similar in all other locations. In all locations and depths, both the variation in the orientation angles and degree of anisotropy of the collagen type I network did not change over time.

Figure 3 shows representative examples of PLM images of the collagen network.

4 | DISCUSSION

This study provides further insight into the post-natal bone development of the precocial foal, which can be seen as a reflection of the strategies of how this precocial animal copes with the mechanical loading associated with locomotion. The equine tarsal joint is loaded in multiple directions, as weight bearing of the hindlimb leads to tensile strain of the lateral part and compression of the medial part of the talus (Schneider, Milne, Gabel, Groom, & Bramlage, 1982). Additionally, simultaneous shear and compressive forces are exerted on the talus (Badoux, 1987), as the slanting talar trochlear ridges tightly interlock in the tibial cochlea to stabilise the tarsus (Nickel, Schummer, & Seiferle, 2003). Whereas absolute forces obviously increase with growth, relative loading remains stable in the

early juvenile phase, as body weight-normalised peak vertical forces at walk and trot stay relatively constant during the first half year of the foal's life (Gorissen et al., 2017).

The absence of a reduction in trabecular BV/TV after birth in foals as seen in this study, and of the increase in BV/TV in the following months, mirrors the gradual increase in BV/TV after the onset of walking in altricial species (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006; Wolschrijn & Weijs, 2004). The increase in trabecular BV/TV is caused by the significant increase in Tb.Th, which is a common hallmark of trabecular bone growth and reported in many other species and skeletal locations (Gosman & Ketcham, 2009; Mulder, Koolstra, Weijs, & Van Eijden, 2005; Ryan & Krovitz, 2006; Wolschrijn & Weijs, 2004). A possible explanation for the lower BV/TV values observed in babies and puppies at the onset of walking compared to the foal may be that in the altricial species, the process of learning to walk is much more gradual, whereas foals need to be able to outrun predators from day 1. The increase in trabecular BV/TV is also reflected by the increase in collagen type I network. Optical retardance is dependent on the amount of birefringent material and its anisotropy (Rieppo et al., 2007). As the anisotropy of the trabecular bone did not change significantly over time, the increase in retardation is due to an increase of collagen.

In foals, Tb.N. stays relatively constant after birth, which is in contrast to reports in altricial (Wolschrijn & Weijs, 2004) and semi-precocial (Tanck et al., 2001) animals and in humans (Gosman & Ketcham, 2009; Ryan & Krovitz, 2006). According to the Mechanostat theory (Frost, 2001) and Wolff's law of bone adaptation (1892), this would indicate that in the foal after birth no trabeculae are loaded under their threshold, which would lead to resorption. This observation supports the idea of pre-natal anticipatory bone development, as proposed earlier (Gorissen et al., 2016). Further support for this concept can be found in the relative constant orientation of both trabeculae and collagen network. These orientations are highly correlated with the main direction of loading (Mittra, Rubin, & Qin, 2005; Silva et al., 2006; Ulrich, Hildebrand, Van Rietbergen, Müller, & Rüegsegger, 1997; Wang, Bank, TeKoppele, & Agrawal, 2001), and hence, the lack of changes means that both are already in line with post-natal loading with form preceding function, in contrast to Wolff's paradigm. A comparable situation has been shown to exist in equine articular cartilage where an anisotropic collagen network was already evident prior to birth (Cluzel, Blond, Fontaine, Olive, & Laverty, 2013). Also in the subchondral bone layer, the basic collagen framework seems to have attained the mature configuration at an age of 5 months (Holopainen et al., 2008). This may seem extremely early given the fact that horses reach skeletal maturity (as defined by growth plate closure) at an age of 4 years (Kainer, 1987), but it is questionable whether growth plate closure is the event to relate to. A relation with the maturation process of the collagen network in the articular cartilage may be more logical, as this structure is functionally much more closely related to the subchondral bone than the growth plates. In foals, the functional adaptation of articular cartilage indeed essentially takes place as early as during the first

(a) Subchondral bone volume fraction

(b) Subchondral bone density

(c)
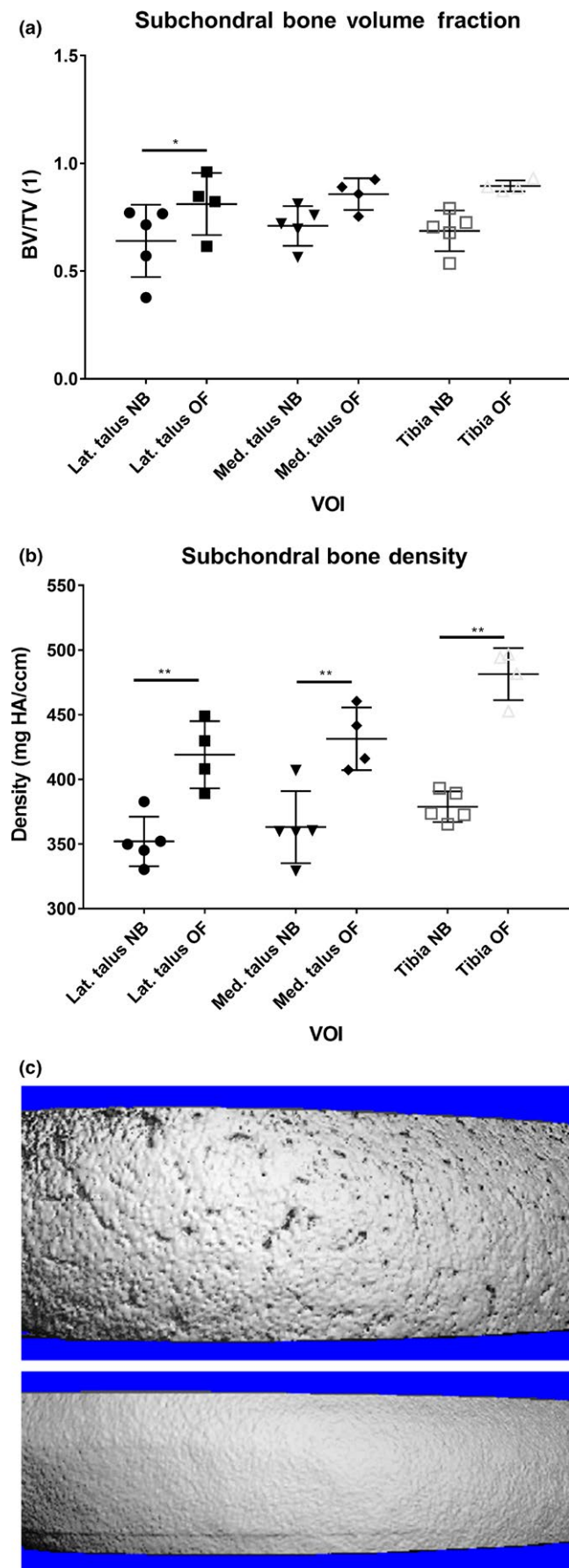
FIGURE 2 Mean (±*SD*) subchondral bone parameters calculated for the different volumes of interest (VOIs, Lat. talus: lateral part of the talus; Med. talus: medial part of the talus; Tibia: sagittal ridge of the tibia) and age groups (NB: newborn foals; OF: older foals). *.01 < *p* < .05; **p* < .01. (a) Bone volume fraction (BV/TV). (b) Density. (c) Part of the reconstructed subchondral bone layer of the right lateral trochlear ridge of the talus of a newborn (upper part) and 145-day-old foal (lower part)

5 months of life (Brama, TeKoppele, Bank, Barneveld, & Van Weeren, 2002).

Osteocytes sense the skeleton's mechanical environment by load-driven changes in the fluid flow through the lacunar–canalicular system (You et al., 2008), leading to the secretion of signalling factors, of which nitric oxide is one of the most important (Klein-Nulend et al., 1998; Pitsillides et al., 1995; Westbroek et al., 2000). These signalling substances influence bone mass, mineral density and matrix formation to match form and function of the skeleton and thereby guide skeletal remodelling. Although the process of mechano-sensing in the developing skeleton is largely unexplored, it is known that the phenomenon exists and that loading is essential for normal joint and bone development. Individuals with congenital neuromuscular disorders are known to suffer from joint contractures and to have bone with thinner cortexes (Fanconi, Ensner, & Knecht, 1995; Rodríguez & Palacios, 1989). Furthermore, experiments with in ovo- or in utero-immobilised chicken and mice have shown that muscle contraction is important for the development of bony ridges and entheses (Blitz et al., 2009; Tatara et al., 2014) and for limb proportionality (Pollard, McGonnell, & Pitsillides, 2014). The need for intrauterine loading seems to differ with the anatomical location. In a mouse model using mice with disrupted skeletal muscle development, thus lacking mechanical stimulation from embryonic muscle contractions, bone development was affected in the scapula, humerus, ulna, femur and elbow joint. However, the tibia and knee joints developed normally, indicative of location-specific regulation of bone and joint development (Nowlan et al., 2010). The exact underlying mechanism remains elusive, but possibly intrauterine motion might be sufficient to start a load history resulting in the functional adaptation prior to birth documented in this paper. In that context, it would be interesting to study other, more proximal locations in foals, known to develop abnormally in the affected mouse model, to see whether bone development in these regions is comparable with the more distal tibia and talus.

In line with previous studies (Wolschrijn & Weijs, 2004), the subchondral bone layer in the foal was shown to develop in the form of patches, which first appeared in those locations with the highest loading. At birth, substantial variation was found in the composition and structure of this layer, as reflected by the differences in BV/TV. It can be hypothesised that there might be a relationship with the susceptibility to osteochondrosis. Osteochondrosis (OC) is a disorder of the process of endochondral ossification as this takes place

TABLE 1 Mean (±*SD*) polarised light microscopy (PLM) parameters calculated for the different volumes of interest (VOIs), age groups and depths (1 = superficial, 3 and 4 = deepest)

		Average retardation					Standard deviation retardation			
		Newborn foals		Older foals			Newborn foals		Older foals	
	Depth	Mean	SD	Mean	SD	Depth	Mean	SD	Mean	SD
Lateral part of the talus	1**	4.0584	2.1600	11.0463	1.6888	1**	2.1876	1.2943	5.7574	0.9316
	2**	3.8181	1.9820	11.4547	2.0228	2**	2.1153	1.1483	5.7964	0.9280
	3**	3.9024	2.0439	11.6886	2.1248	3**	2.1218	1.2185	5.8980	1.0067
	4**	4.0254	2.0506	11.2537	2.6871	4**	2.1954	1.1145	5.6164	1.1952
Medial part of the talus	1**	6.0745	3.1144	11.2477	2.1300	1*	3.7726	1.5588	5.8482	1.2020
	2**	7.6087	2.4240	12.8514	3.0764	2**	4.2346	1.2339	7.2081	3.3001
	3**	7.0928	1.2646	12.0382	2.1419	3**	3.5084	0.9299	6.2624	1.2997
	4*	6.2402	1.3375	10.5530	2.4651	4**	2.9334	1.1243	5.0454	1.4340
Tibia	1**	5.6093	3.0609	12.9550	2.5087	1**	3.1800	1.5663	6.9114	2.4877
	2**	6.6327	1.7825	12.5324	1.6876	2**	4.0080	0.9164	6.6573	1.9330
	3**	7.1468	1.6784	12.1337	2.6464	3**	3.9779	1.0098	6.0049	1.6362
		Average orientation					Standard deviation orientation			
		Newborn foals		Older foals			Newborn foals		Older foals	
	Depth	Mean	SD	Mean	SD	Depth	Mean	SD	Mean	SD
Lateral part of the talus	1	49.4749	11.0144	42.7368	2.5858	1	23.4554	2.9415	25.5006	0.3553
	2	45.7456	7.8133	41.0646	2.0658	2	24.9643	1.3633	25.4046	0.3633
	3	45.4571	3.6816	42.5746	2.1579	3	25.9176	0.3069	25.6996	0.2997
	4	47.1897	2.7361	45.6648	1.6902	4	25.7958	0.4835	25.6693	0.2007
Medial part of the talus	1	38.4557	4.4079	41.1603	4.8405	1	25.3196	0.9501	25.3270	0.5915
	2**	38.6081	7.7788	49.3621	4.1727	2	24.4817	1.8609	24.7602	0.8419
	3**	40.6235	7.8532	51.6843	3.7798	3	24.8268	1.2102	24.6841	0.3551
	4**	42.1042	9.1937	52.1770	4.6046	4	24.8416	0.9979	25.1162	0.7649
Tibia	1	40.3431	3.6003	44.3497	4.0831	1	24.9698	1.1057	25.7129	0.6809
	2	42.6789	3.8052	38.5930	4.0845	2	25.7780	0.3845	25.2556	0.8264
	3	45.1640	2.3641	43.8060	4.0680	3	25.8318	0.2905	25.2954	0.9328

	Degree of anisotropy				
		Newborn foals		Older foals	
	Depth	Mean	SD	Mean	SD
Lateral part of the talus	1	0.1369	0.0043	0.1336	0.0002
	2	0.1347	0.0021	0.1339	0.0003
	3	0.1337	0.0004	0.1336	0.0003
	4	0.1337	0.0005	0.1335	0.0002
Medial part of the talus	1	0.1347	0.0016	0.1343	0.0009
	2	0.1355	0.0031	0.1344	0.0011
	3	0.1351	0.0025	0.1349	0.0008
	4	0.1352	0.0020	0.1356	0.0014
Tibia	1	0.1344	0.0009	0.1339	0.0008
	2	0.1337	0.0004	0.1346	0.0012
	3	0.1336	0.0002	0.1344	0.0011

*.01 < p < .05; **p < .01.
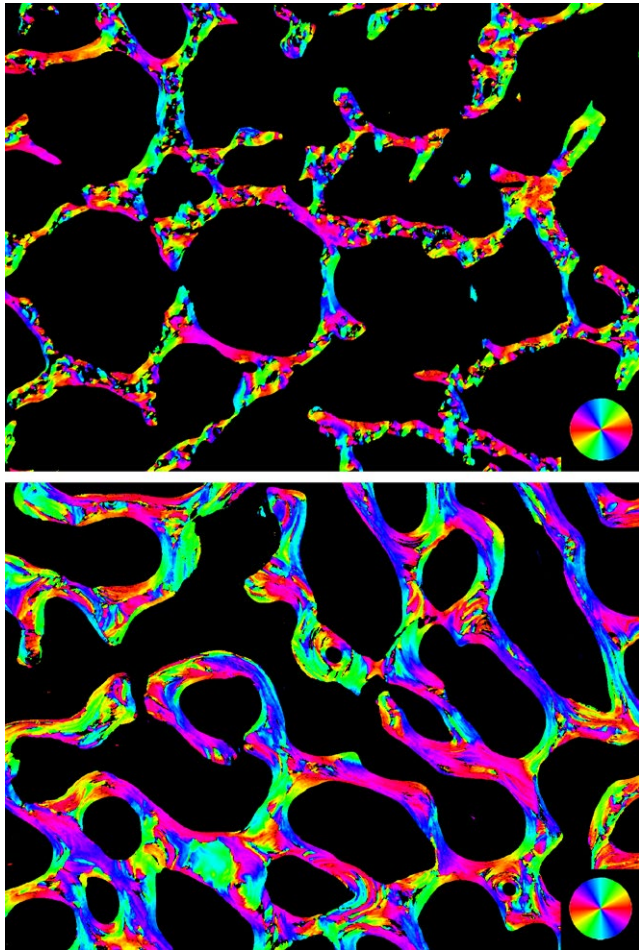
FIGURE 3 Representative example of a polarised light microscopy (PLM) image from the trabecular bone of the distal part of the lateral trochlear ridge of a newborn (upper part) and older (145 days old; lower part) foal. The different colours represent the orientation of the collagen type I network as indicated in the legend in the right-hand corner

5 | CONCLUSION

We confirmed in this study that in contrast to more altricial species, foals have an anticipating way of bone development, with respect to both composition (BV/TV) and architecture (trabecular DA and collagen orientation). These observations support and expand earlier observations pointing at the existence of such a pre-natal anticipatory bone development (Gorissen et al., 2016) and demonstrate the strategy followed by the horse to cope with the extreme changes in mechanical environment that the locomotor system experiences around birth. Furthermore, they can be helpful in better understanding the aetiology of development of orthopaedic diseases.

CONFLICT OF INTEREST

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at the articular side of the epiphyseal ossification centre (Ytrehus, Carlson, & Ekman, 2007). Although foals are able to follow their dam from day 1, the development of static balance (Nauwelaerts, Malone, & Clayton, 2013) and gait (Gorissen et al., 2017) takes time, which increases the risk of stumbling and consequently the generation of focal peak loads in joints. It can be conjectured that foals featuring biomechanically less favourable subchondral and trabecular bone parameters at birth would be more susceptible than others to sustaining vascular damage and consequently develop OC due to hypoxia of the chondrocytes.

Due to the difficulty in obtaining material from foals meeting the inclusion criteria, the number of animals included in this study was limited, which is considered a limitation. Nevertheless, all animals had developed normally until death or euthanasia and can be seen as representative for the Dutch warm-blooded breed. All also showed similar developmental patterns with regard to the variables investigated in this study.

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