Can bone measures of the bovine metacarpus predict humeral bone structure?

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Abstract

Peak bone mass in cattle is influenced by factors prior to puberty and insufficient peak bone mass prior to the first lactation predisposes heifers to the risk of spontaneous humeral fractures. At post-mortem examination, affected heifers present with osteoporosis (reduced periosteal circumference and reduced cortical wall thickness). Due to its location, there is no cost-effective way to scan the humerus in the live animal. However, the metacarpal bones (MC3/MC4) are straightforward to scan in a live anaesthetised animal. The aim of this experiment was to determine if the metacarpal could be used to predict bone mass and material properties of the humerus. The left humerus and metacarpal bones were obtained from 57 cattle aged six weeks to eight years. Bones were scanned at the mid-diaphysis using peripheral quantitative computed tomography (pQCT), to obtain measures of bone mass and material properties (strength). Strong positive relationships were observed (R^2=0.89 - 0.98, $P<0.0001$) for pQCT measurements of bone mass and strength. Thus, the metacarpus is a good predictor of bone content and material properties of the humerus across cattle of differing age and maturity.

Keywords: Bone; fracture; humerus; metacarpal; dairy heifer; bone strength

Introduction

Spontaneous humeral fracture is an emerging condition affecting at least 4% of all dairy farms in New Zealand. It is predicted that approximately 5,000 heifers per year are lost to this syndrome, resulting in an economic loss of around $10 million to the New Zealand dairy industry (Dittmer et al. 2016). Currently there is no method to identify heifers that are at risk of humeral fractures. At present, a herd can only be identified as being at risk of humeral fractures when multiple heifers have been affected, at which point there is limited opportunity to increase bone quality and quantity on a farm level. Post-mortem examination of humeral fractures has identified reduced cortical bone thickness in affected bones (Dittmer et al. 2016).

To understand why the humerus, in particular, appears to be most at risk of fracture, bone developmental stages need to be considered. This includes changes in bone measurements with growth and nutritional factors when peak bone mass occurs. Humeral growth is constant with growth in size of the animal, whereas bones in the distal limb, such as the third and fourth metacarpal (MC3/MC4), have a decrease in growth trajectory as the animal gets larger. Berg et al. (1978) compared the proportion of bone to total bone weight of carcasses reported in different studies and demonstrated that as the size of the carcass increased, the proportion of metacarpal weight to total bone weight decreased whereas, the proportion of bone in the shoulder (including the humerus) remained constant (Berg et al. 1978). This shows how the humerus is dynamic in response to growth, while the metacarpus is less so.

The majority of peak bone mass is accumulated at puberty. Just prior to puberty, there is a rapid increase in bone mass, due to rising secretions of growth hormone (GH), insulin-like growth factor 1 (IGF-I) and sex steroids (Glastre et al. 1990). The increase in circulatory GH and IGF-I concentrations promote bone turnover by stimulating osteoblast proliferation and differentiation (Green et al. 1985). Precursors for male and female sex hormones, such as dehydroepiandrosterone, are also related to bone strength. Increases in muscularity at puberty is also thought to be a factor in increasing bone mass during puberty at approximately 12 months old (Gilsanz et al. 2011; Meier et al. 2017). However, growth continues in cattle until around 20-22 months, at which point it slows considerably (Handcock et al. 2018). Therefore, it could be expected that the bone dimensions continue to change based on age and growth until this point.

Bones from cattle with spontaneous humeral fractures have osteoporosis (Dittmer et al. 2016). Deficits in nutrition may cause osteoporosis, and in farm animals in particular, nutrient deficiency associated with starvation is one of the most common causes of osteoporosis (Craig et al. 2016). Inadequate nutrition (total energy and protein requirement) on farm may occur due to drought or overstocking, leading to a lack of pasture, or lack of quality pasture, or both. Young animals are more prone to the effects of undernutrition due to the energy requirements of growth and development, for example in dairy calves when milk quality or quantity is limited. Cooper et al. (1997) reported that human infants with low birth weights had a reduced bone mineral content at an older age compared to bone mineral content in human infants of a normal birth weight. This could be a possible risk factor for humeral fractures, when calves fed restricted diets early in life could have lower bone mineral content and, therefore, be at risk of fracture.

Bone composition can be measured using peripheral quantitative computed tomography (pQCT), providing quantitative data on trabecular and cortical bone material properties and distribution (Firth et al. 2011). In the anaesthetised animal, pQCT of the distal limb can be performed quickly and reliably. Scanning of distal limb, such as the metacarpus, can be achieved with pQCT in live cattle and in the humerus from animals submitted for post-mortem examination. To interpret any differences in bone composition seen on pQCT, an understanding of normal bovine bone structure and the effects of age is required.

Due to the location of the humerus, the ability to scan the bone in a cost-effective manner is difficult. Scanning of the humerus is only possible at post-mortem when the bone can be removed. This makes it difficult to predict heifers at risk of humeral fracture. It is possible that bones such as the metacarpus that are easily accessible in a live animal, could act as a proxy to predict humeral structure. Therefore, the aim of this study was to determine if pQCT measures of the metacarpal bone could be used to predict the structure of the humerus.

Materials and methods

The metacarpus and corresponding humerus were collected opportunistically from a total of 57 animals from three different studies. As well as bone scans and size, weight, age, and general breed type were recorded. The bones can be divided in to three cohorts. The first cohort (n=17) of bones were collected from a dairy-calf-nutrition study in which calves were fed either 5 L or 10 L of milk a day, and euthanized either at 6 weeks or 12 weeks of age. The second cohort (n=17) of bones was collected from a terminal study in which beef/dairy cross steers were sent to the abattoir at one year of age. The third cohort (n=23) of bones were collected from animals of mixed age submitted for post-mortem examination for non-orthopaedic issues.

Metacarpal length was measured with a ruler, and determined as the distance between the lateral aspect of the lateral condyle of the MC4 and the proximal aspect of the lateral MC4. Humeral length was measured from the proximal end of the humeral head at the lateral aspect to the end of the trochlea at the distal end using a box ruler.

The mid-diaphysis of each bone was scanned using peripheral quantitative computed tomography (pQCT; XCT 2000, Stratec Medical) with the bone being scanned at 50% of the total bone length starting from the distal end with a voxels size of 0.3 mm^3. Voxels were assigned, within the manufactuers software, as either "trabecular" bone (≤710 mg/mL), or as "cortical" bone (>710 mg/mL). Data derived from the scan included measures of total bone content, cortical and subcortical bone content, cortical and subcortical density, trabecular content, trabecular density, total area, trabecular area, cortical and subcortical area, cortical content, cortical density, cortical area, cortical thickness, periosteal circumference and endosteal circumference.

Statistical analysis

Data are presented as least-square means ± SEM by bone, study and parameter. A General Linear Model was used to generate LS means and regression coefficients for bone parameters. The model used the fixed effect of cohort. Where a cohort effect was significant ($P<0.05$), the interaction of cohort and bone was fitted and separate regression equations were produced for each cohort by bone. All statistical analyses were conducted using the Statistical Analysis System software version 9.4 (SAS Institute Inc., Carey NC, USA).

Results

The age and weight range for the calf cohort was 11 to 90 days and 35.5 to 99.5 kg, respectively. All animals in the yearling cohort were approximately one year old and ranged from 303 kg to 392 kg. The mixed-age cohort ranged from two years to eight years of age, but live weights in this cohort were not routinely recorded.

At the mid-diaphyseal site of the metacarpus (MC3/4) there were no differences ($P>0.05$) among cohorts for three of the parameters measured; trabecular bone mineral content and trabecular bone mineral density (Table 1). There were significant differences among age cohorts for the all the remaining bone parameters listed in Table 3. These were associated with significant differences ($P<0.05$) between the calves and the older cohorts of yearlings and mixed-age cows. The yearlings and mixed-age cows had similar values for many of these parameters.

At the mid-diaphyseal site of the humerus there were significant differences for all parameters, except trabecular bone mineral density. In contrast to the metacarpal, the general pattern for the humerus was the presence of significant differences in parameters between all age cohorts.

Strong correlations were observed between the mid-diaphyseal measurements of the humerus and metacarpal (R^2=0.66-0.97, $P<0.001$) for periosteal circumference, total bone mineral content, cortical and subcortical-bone mineral content, cortical and subcortical-bone mineral density, total area and cortical and subcortical-bone area, cortical bone mineral content, cortical bone area, cortical thickness and stress strain index (an index based on the summative weighting of voxel BMD and the square of its location relative to the central axis) (SSI; Table 2). Weak correlations (R^2=0.24-0.46; $P<0.001$) existed for trabecular bone mineral content, trabecular bone mineral density, trabecular bone area and endosteal circumference, whilst there was no correlation for trabecular bone mineral density between the metacarpal and the humerus ($P>0.05$).

Several parameters, generally those associated with size measurements rather than material properties, had high R^2 values, indicating that the metacarpus could be a suitable predictor of the same parameter in the humerus (Table 3). Despite the different intercept, there was no interaction ($P>0.05$) of metacarpal parameters with cohort, so that the relative differences between the bones for these parameters were consistent among ages.

An interaction of cohort and bone was observed for cortical and subcortical bone mineral density and trabecular bone mineral density (Table 4). This resulted in the generation of different regression coefficients for each

Table 1 Composition of study population showing the calves, yearlings and mixed-age cohorts with their average age, weight with standard deviations and main production type for each cohort. Means of bone measures and standard error of mean by cohort and bone.
*Mixed age cows at the time of post-mortem were not routinely weighed.

	Calves	Yearlings	Mixed-age	P value
Cohort				
N	17	17	24	
Mean age	61±6 days	1 Year	4.4±0.5 years	
Mean weight*	68.6±4.6 kg	345.8±5.1 kg	-	
Main production type	Dairy	Dairy/Beef	Dairy	
Metacarpal				
Bone length (mm)	174.8±2.4[a]	207.9±2.8[b]	206.0±2.4[b]	<0.001
Total bone area (mm^2)	345.9±19.1 [a]	759.7±19.1 [b]	727.8±16.14 [b]	<0.001
Total bone content (mg/mm)	254.4 ± 15.3 [a]	679.0 ± 15.3 [b]	695.9±13.1 [b]	<0.001
Periosteal circumference (mm)	65.7±1.4 [a]	97.6±1.4 [b]	95.4±1.2 [b]	<0.001
Endosteal circumference (mm)	43.7±1.9[a]	55.2±1.9[b]	50.4±1.6[b]	<0.001
Cortical thickness (mm)	3.5±0.2[a]	6.8±0.2[b]	7.2±0.1[b]	<0.001
Cortical bone area (mm^2)	193.5±10.4 [a]	513.0±10.4 [b]	518.8±8.9 [b]	<0.001
Cortical bone content (mg/mm)	223.4±15.9 [a]	646.2±15.9 [b]	663.9±13.7 [b]	<0.001
Cortical bone density (mg/cm^3)	1152.6±11.3[a]	1257.0±11.3[b]	1278.9±9.7[b]	<0.001
Cortical/ subcortical bone area (mm^2)	222.1±10.4 [a]	542.5±10.4 [b]	543.8±9.0 [b]	<0.001
Cortical/subcortical bone content (mg/mm)	241.0±15.8 [a]	659.6±15.8 [b]	675.3±13.5 [b]	<0.001
Cortical/subcortical density (mg/cm^3)	1081.9±12.6 [a]	1213.5±12.6 [b]	1241.6±10.8 [b]	<0.001
Trabecular bone area (mm^2)	123.9 ± 14.8 [a]	217.1 ± 14.8 [b]	184.0 ± 12.8 [b]	<0.001
Trabecular bone content (mg/mm)	13.4±2.7	19.4±2.7	21.0±2.3	0.092
Trabecular density (mg/cm^3)	113.0±8.4	92.3±8.4	109.6±7.2	0.154
Stress strain index (mm^3)	1060.0±113.9[a]	4536.0±134.7[b]	4445.9±115.8[b]	<0.001
Humerus				
Bone length (mm)	211.6±2.8[a]	240.3±2.8[b]	265.5±2.4[c]	<0.001
Total bone area (mm^2)	634.9±42.3 [a]	1522.7±42.3 [b]	1808.5±36.4 [c]	<0.001
Total bone content (mg/mm)	386.3±30.0 [a]	1266.5±30.0 [b]	1429.0±25.8 [c]	<0.001
Periosteal circumference (mm)	88.7±2.1 [a]	138.2±2.1 [b]	150.5±1.8 [c]	<0.001
Endosteal circumference (mm)	65.24±2.24 [a]	84.2±2.24[b]	98.4±2.0 [c]	<0.001
Cortical thickness (mm)	3.71±0.18 [a]	8.6±0.18 [b]	8.3±0.16 [b]	<0.001
Cortical bone area (mm^2)	288.65±22.23 [a]	953.8±22.23 [b]	1030.5±19.1 [c]	<0.001
Cortical bone content (mg/mm)	339.01±29.20 [a]	1202.8±27.6[b]	1351.3±23.6 [c]	<0.001
Cortical bone density (mg/cm^3)	1174.19±6.67[a]	1262.5±6.67[b]	1310.3±5.73[c]	<0.001
Cortical and subcortical bone area (mm^2)	323.5±23.3 [a]	996.4±23.3 [b]	1076.0±20.1 [c]	<0.001
Cortical/Subcortical bone content (mg/mm)	356.0±29.6 [a]	1222.0±29.6 [b]	1369.6±25.4 [b]	<0.001
Cortical/subcortical density (mg/cm^3)	1099.1±7.2 [a]	1228.0±7.2 [b]	1272.0±6.2 [c]	<0.001
Trabecular bone area (mm^2)	311.4±30.2 [a]	526.3±30.2 [b]	732.5±26.0 [c]	<0.001
Trabecular bone content (mg/mm)	30.2±3.0 [a]	44.5±3.0 [b]	59.4±2.6 [c]	<0.001
Trabecular density (mg/cm^3)	100.5±4.8[a]	85.5±4.8[b]	81.7±4.1[b]	0.012
Stress strain index (mm^3)	836.3±476.5[a]	12878.7±476.5[b]	15121.6±409.7[c]	<0.001

age group. The yearling cohort had the least change in the humerus relative to the metacarpus compared to the mixed-age and calf cohorts, as shown by the low coefficient for all four traits. The mixed-age cohort had the largest difference between the two bones, which is represented by large coefficients in the regression equation.

Discussion

Simple correlations comparing the mid-diaphysis of the metacarpal and humeral bones demonstrated that the metacarpal is a good predictor of humeral bone structure in cattle. The relationships were strongest with the cortical bone and total bone area parameters.

With increasing age, there was an increase in periosteal circumference, but this was not associated with the same relative increase in cortical wall thickness, and the rate of increase in cortical wall thickness was lower in the humerus than in the metacarpus. This is likely to be associated with bending strain associated with the different anatomical locations of the bones (proximal versus distal) and the relatively greater increases in bending strength obtained by increasing bone circumference and cortical wall thickness (van Der Meulen et al. 1993). This observation was supported by the stress strain index, where the SSI of the bone increased with increased mean periosteal circumference between the yearling and mixed-age cohorts, despite no corresponding increase in cortical thickness.

The SSI was approximately three times higher in the humerus than in the metacarpus in the yearling and mixed-age cohorts. Bone is dynamic and changes in response to increases in loading by increasing bone strength (the mechanostat theory). A larger SSI in the humerus, and hence greater resistance to bending strain, implies that the mechanical forces that act on the humerus are greater than those acting on the metacarpus, especially in older cattle. This may explain why reduced cortical thickness and periosteal circumference predispose dairy cattle to spontaneous humeral fractures, but not fractures of distal limb bones such as the metacarpus.

Table 2 Correlations between bone parameters in the metacarpal with the same measure in the humerus in the calf, yearling and mixed-age cohorts.

	Correlation	P value
Total bone area	0.89	<0.001
Total bone content	0.96	<0.001
Periosteal circumference	0.92	<0.001
Endosteal circumference	0.46	<0.001
Cortical bone thickness	0.91	<0.001
Cortical bone area	0.96	<0.001
Cortical bone content	0.95	<0.001
Cortical bone density	0.66	<0.001
Cortical/subcortical bone area	0.95	<0.001
Cortical/subcortical bone content	0.95	<0.001
Cortical/subcortical density	0.73	<0.001
Trabecular bone area	0.45	0.001
Trabecular bone content	0.24	0.071
Trabecular bone density	0.24	0.006
Stress Strain Index	0.97	<0.001

Mean bone parameters of the metacarpus in the yearling cohort and mixed-age cohort were not significantly different. However, in the humerus, the mixed-age cohort had significantly higher bone parameters compared to yearlings. Development in the metacarpus, is, therefore likely to be completed by one year of age, while the humerus continues to develop. This is in agreement with Pomeroy (1978) who suggested that the development of upper limbs such as the humerus occurs later compared with the metacarpus. The continuing development of the humerus after one year of age shows how dynamic the humerus can be in response to body growth and increases in muscular forces placed on the bone.

Conclusion

To conclude, pQCT scans of the metacarpus allow for predictions of humeral structure in cattle, possibly enabling the detection of animals at risk of humeral fractures and providing an opportunity for preventative measures to be taken.

Acknowledgements

The authors acknowledge Massey University for their funding and the staff of Massey University post-mortem lab and Number One Dairy Farm for their assistance in collecting samples.

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Table 3 Regression equations for prediction of the same bone parameter in the humerus where there was no study interaction in dairy cattle in the calf, yearling and mixed-age cohorts.

	Intercept calves	Intercept yearlings	Intercept mixed-age	Coefficient all studies	R^2
Total area	237.58	650.23	972.63	1.15	0.92
Total bone content	135.27	596.5	742.32	0.99	0.95
Periosteal circumference	26.62	45.9	60.31	0.95	0.94
Endosteal Circumference	43.41	56.62	73.20	0.5	0.75
Cortical bone thickness	2.30	5.88	5.42	0.40	0.91
Cortical bone area	93.91	580.39	509.5	1.0	0.95
Cortical bone content	150.17	656.67	790.22	0.85	0.95
Cortical bone density	1229.76	1323.08	1371.9	-0.048	0.82
Cortical/subcortical bone area	99.37	448.87	527.21	1.01	0.94
Cortical/subcortical bone area	99.38	448.86	527.21	1.01	0.94
Trabecular bone area	215.05	357.42	589.38	0.78	0.72
Trabecular bone content	28.92	42.56	57.4	0.1	0.50
Stress strain index	-974.2	-1356.6	1169.0	3.1	0.98

Table 4 Regression equations where there was a study effect for prediction of the humeral cortical and subcortical density and trabecular bone density in dairy cattle in the calf, yearling and mixed-age cohorts.

	Calves		Yearlings		Mixed age		
	Intercept	Coefficient	Intercept	Coefficient	Intercept	Coefficient	R^2
Cortical/subcortical density	1509.14	486.01	1189.55	-0.19	0.57	0.03	0.88
Trabecular bone density	79.17	29.43	77.0	0.02	0.63	0.09	0.34

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