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The Mid-Diaphysis Is a Poor Predictor of Humeral Fracture Risk Indicating That Predisposing Factors Are Recent

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Abstract: The incidence of spontaneous humeral fractures in first-lactation dairy heifers in New Zealand has emphasised the need to understand the thoracic limb bone growth of dairy heifers. Previous research has indicated that a predisposing factor to spontaneous humeral fracture is nutrition. In addition, it has been hypothesised that liver copper concentration affects bone strength and may be a potential factor associated with humeral fracture risk. The aim of this study was to compare bone morphology in the mid-diaphysis of the metacarpus and humerus of heifers affected and unaffected by spontaneous humeral fractures, and determine the effect of copper status at death on bone morphology. The metacarpus and humerus were collected from heifers affected and unaffected by humeral fractures, and scanned using peripheral quantitative computed tomography (pQCT). The mid-diaphysis of the humerus of the affected group had reduced cortical bone mineral density and a trend for reduced cortical content and total bone content, which contributed to a reduced stress–strain index. The trend for reduced bone length in affected humeri provides additional support for the hypothesis of inhibited humeral growth. Heifers with low copper liver concentrations had reduced humerus lengths and reduced cortical bone mineral densities. These data support the hypothesis that the developmental window for humeral fracture is recent, and possibly associated with periods of inadequate nutrition.

Keywords: bone strength; fracture; humerus; metacarpal; pQCT; mid-diaphysis

1. Introduction

Spontaneous humeral fracture in first-lactation dairy heifers is an established condition affecting at least 4% of all dairy farms in New Zealand. It is predicted that approximately 5000 heifers per year are lost to this syndrome, resulting in significant economic loss to the New Zealand dairy industry (J. Hunnam, unpublished data).

Humeral fractures in cattle tend to be spiral fractures that originate from the proximal end of the diaphysis [1]. In cattle with normal cortical bone thickness, humeral fractures are rare (5% of all fractures in cattle and 18% of all long bone fractures) due to the protection provided by the biceps brachii, brachiocephalicus, and brachialis muscles that surround the humerus caudally, laterally and cranially [1]. In contrast, spontaneous humeral fractures in first-lactation dairy heifers are reported to originate from the distal metaphysis [2]. Post-mortem examinations of spontaneous humeral fractures have shown an association with lower cortical bone thickness in affected bones, contributing to a decrease in bone strength (measured by stress–strain index) [2]. Growth arrest lines have also been observed in the humeri from heifers with spontaneous humeral fractures, indicating a period of malnutrition at some point in the heifer's life [2]. The combination of weaker (thinner) bones and the sudden draw of calcium due to the onset of lactation is likely to contribute to

an increased risk of spontaneous fractures in the humerus. Additionally, copper deficiency, which is common in New Zealand cattle, is known to affect bone strength and can cause osteoporosis in severely deficient animals [2]. At present, it is unknown if copper deficiency is a contributing factor to fracture risk in first-lactation dairy cattle.

The identification of heifers at risk for humeral fracture is difficult due to the location of the humerus. The ability to examine the bone is restricted to post-mortem examination. Therefore, a herd can only be identified as being at risk of having a higher number of animals with humeral fractures when multiple heifers have been affected, at which time there is limited opportunity to provide remedial management strategies to increase bone strength. However, the mid-diaphysis of the metacarpus is easily accessible, and has been shown to have bone parameters that are highly correlated with those of the humerus [3]. Peripheral quantitative computed tomography (pQCT) allows for the quantification of bone morphology in both the metacarpus and humerus. However, due to the large size of the humeral head and small size of the gantry hole in the scanner, scanning is restricted to the mid-diaphysis. Currently, there are minimal CT data on bone morphology in the humerus of cattle, apart from the studies of Bouza-Rodríguez and Miramontes-Sequeiros [4], who developed a finite element model of the humerus and identified that the distal metaphysis is the weakest site. The incidence of spontaneous humeral fractures in first-lactation dairy heifers has prompted the need to examine the humerus. Therefore, the aim of the study was to compare bone morphology in the mid-diaphysis of the metacarpus and humerus of heifers affected and unaffected by spontaneous humeral fractures, and determine the effect of copper status at death on bone morphology.

2. Materials and Methods

2.1. Sample Collection

2.1.1. Control Group

A collection of 29 metacarpals and 30 humeri (29 sets) was obtained from 2-year-old dairy cattle that died from non-bone-related issues. Bones were collected from a casualty cow collection service (Wallace Corporation, Feilding) that was visited for sample collection twice a week during October 2020. Additional bones were collected from Massey University's post-mortem facility. Any cows with an ear tag indicating they were born in 2018 and an udder consistent with lactating at the time of death were selected. When possible (n = 16), a liver sample was collected and analysed for copper concentration using inductively coupled plasma mass spectrometry (ICP-MS), at New Zealand Veterinary Pathology (IDEXX) Ltd. (Palmerston North, New Zealand). Copper was categorised as normal (>95 μmol/kg), low (30–95 μmol/kg) and very low (<30 μmol/kg). A liver copper concentration of below 30 μmol/kg has been associated with impaired animal growth in cattle [5].

2.1.2. Affected Group

In total, 86 metacarpals and 93 contralateral humeri (83 sets) from dairy cattle that had suffered humeral fractures were collected, through the help of veterinarians and farmers around New Zealand, during the 2019/2020 dairy season. Bones were collected by veterinarians and farmers following researcher requests via social media and email. Bones were sent disarticulated via courier and stored at −20 °C until scanning. Where possible medical records were requested, but as a general rule these were limited and brief. Liver samples were also collected for 69 of the cases and analysed for copper concentration using inductively coupled plasma mass spectrometry (ICP-MS), at New Zealand Veterinary Pathology (IDEXX) Ltd. (Palmerston North, New Zealand). Copper was categorised as normal (>95 μmol/kg), low (30–95 μmol/kg) and very low (<30 μmol/kg). A liver copper concentration of below 30 μmol/kg has been associated with impaired animal growth in cattle [5].

2.2. Scanning

pQCT scanning was carried out using the same protocol previously reported by Gibson, et al. [6] and Gibson, et al. [7]. Briefly, pQCT scanning was carried out using an XCT 2000 peripheral quantitative computed tomography machine (Stratec Medical, Pforzheim, Germany). For each bone, a 2 mm slice at the mid-diaphysis was obtained with a voxel size of 0.3 mm. In the metacarpus, the mid-diaphysis was defined as 50% of the total bone length. Length was measured using the lateral aspect of the lateral condyle of the MC4 and the proximal aspect of the lateral MC4. In the humerus, the mid-diaphysis was defined as 50% of total bone length. 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. Within the manufacturer's software, voxels > 710 mg/cm^3 CaHA were assigned as "cortical" bone. Data derived from the scan included measures of total bone content (>710 mg/cm^3), cortical density, total area, cortical area, cortical content, cortical thickness, periosteal circumference, endosteal circumference and stress–strain index (SSI). The stress–strain index is a measure of bone strength derived using pQCT data and avoids the requirement for destructive testing using techniques such as the three-point bending test. The stress–strain index describes the ability of bone to withstand bending from lateral, dorso-palmar and torsional forces, and is calculated by incorporating the indexes of material stiffness (bone mineral density) and bone geometry (cross-sectional moment of inertia) [8]. The pQCT variables will be referred to as bone parameters in the statistical models.

2.3. Statistical Analysis

Statistical analysis was conducted using the Statistical Analysis System software version 9.4 (SAS institute Inc., Carey, NC, USA). All analyses were conducted using general linear models.

Data were tested for normality using the Shapiro–Wilk test, which indicated that all bone parameters followed a normal distribution. Data are presented as least-square means and standard errors for each heifer group and bone parameter. Bone parameters were compared between control and affected heifers using a general linear model that included the fixed effect of the group.

In order to adjust for expected mature weight, bone parameters in the humerus were also compared between groups after adjusting for the periosteal circumference of the metacarpus. These models included the fixed effect of group and the covariate of periosteal circumference in the metacarpus (as a predictor of mature size). The interaction of periosteal circumference in the metacarpus and group was considered, but was not significant, so was removed ($p > 0.05$).

Similarly, the effect of copper was tested using a general linear model that included the fixed effect of copper status (normal, low or very low) and the covariate of periosteal circumference in the metacarpus. The fixed effect of group was tested but not significant, so was removed.

3. Results

In the metacarpus, endosteal circumference was greater in the affected heifers than the control heifers (Table 1). Affected heifers had lower cortical bone density (0.9% difference) in both the metacarpus and the humerus than control heifers.

Table 1. Least squared means and standard errors of bone parameters in the metacarpus and humerus of control and affected heifers.

Bone Parameter	Control	Affected	*p*-Value
Metacarpus			
n	29	86	
Bone length (mm)	206.9 ± 1.3	209.3 ± 0.8	0.114
Periosteal circumference (mm)	92.4 ± 1.0	93.9 ± 0.6	0.180
Endosteal circumference (mm)	46.3 ± 1.0	48.7 ± 0.6	0.041
Total bone area (mm^2)	681.4 ± 14.7	704.5 ± 8.5	0.178
Total bone content (mg/mm)	677.9 ± 11.7	677.8 ± 6.8	0.993
Cortical bone thickness (mm)	7.3 ± 0.1	7.2 ± 0.1	0.184
Cortical bone area (mm^2)	508.8 ± 9.0	513.1 ± 5.3	0.681
Cortical bone density (mg/cm^3)	1279.4 ± 2.5	1267.6 ± 1.5	<0.001
Cortical bone content (mg/mm)	651.0 ± 11.4	650.3 ± 6.6	0.955
Stress–strain index (mm^3)	4031.1 ± 126.4	4207.3 ± 73.4	0.231
Humerus			
n	30	93	
Bone length (mm)	258.3 ± 1.9	257.1 ± 1.1	0.575
Periosteal circumference (mm)	143.5 ± 1.6	144.1 ± 0.9	0.753
Endosteal circumference (mm)	93.6 ± 1.5	94.8 ± 0.9	0.501
Total bone area (mm^2)	1644.6 ± 37.5	1657.4 ± 21.3	0.767
Total bone content (mg/mm)	1285.6 ± 26.2	1265.6 ± 14.9	0.508
Cortical bone thickness (mm)	7.9 ± 0.1	7.8 ± 0.1	0.507
Cortical bone area (mm^2)	939.8 ± 20.2	936.4 ± 11.5	0.883
Cortical bone density (mg/cm^3)	1292.6 ± 3.7	1280.4 ± 2.1	0.005
Cortical bone content (mg/mm)	1214.6 ± 25.3	1198.3 ± 14.4	0.578
Stress–strain index (mm^3)	13,335.9 ± 416.1	13,261.3 ± 236.3	0.876

Adjusting humeri measurements with metacarpus periosteal circumference, as a proxy for bodyweight, revealed a pattern of moderate inhibition of longitudinal bone growth and reduced deposition of mineral content in affected heifers. Affected heifers presented with significantly reduced cortical bone density ($p < 0.05$) and a strong trend for reduced total bone content ($p = 0.057$) (Table 2). The lack of significant difference in cortical area indicates that the trend observed in lower cortical bone content was the result of a reduced cortical bone mineral density in affected heifers. The resultant effect of the reduced cortical bone mineral density and the trend in lower total bone content was a trend for lower SSI, as observed in the affected heifers.

Adjusting humeri measurements with metacarpus periosteal circumference, as a proxy for bodyweight, revealed that heifers in the very low liver copper concentration group had reduced humeral bone length (inhibited longitudinal growth) and low cortical bone density (Table 3). The lower cortical bone density was not associated with lower cortical area or content, which may explain the lack of difference identified in SSI.

Table 2. Least squared means and standard errors of bone parameters in the humerus using periosteal circumference of metacarpus as a predictor between control and affected heifers.

Bone Parameter	Control	Affected	Coefficient	MC3 Periosteal Circumference	*p*-Value Group	R^2
n	29	83				
Bone length (mm)	259.7 ± 1.6	256.5 ± 1	1.2 ± 0.2	<0.001	0.094	0.35
Periosteal circumference (mm)	144.7 ± 0.9	143.5 ± 0.5	1.3 ± 0.1	<0.001	0.258	0.67
Endosteal circumference (mm)	94.9 ± 1.2	94.3 ± 0.7	1.0 ± 0.1	<0.001	0.660	0.44
Total bone area (mm^2)	1673.2 ± 21	1643.5 ± 12.4	31.0 ± 2.1	<0.001	0.228	0.67
Total bone content (mg/mm)	1297.1 ± 17.6	1257.7 ± 10.3	19.7 ± 1.7	<0.001	0.057	0.54
Cortical bone thickness (mm)	7.9 ± 0.1	7.8 ± 0.1	0.05 ± 0.01	<0.001	0.495	0.12
Cortical bone area (mm^2)	948.7 ± 13.8	930.8 ± 8.1	14.9 ± 1.4	<0.001	0.267	0.53
Cortical bone density (mg/cm^3)	1291.2 ± 3.7	1280.0 ± 2.2	−1.0 ± 0.4	0.008	0.010	0.13
Cortical bone content (mg/mm)	1224.4 ± 17.8	1191 ± 10.5	18.2 ± 1.7	<0.001	0.110	0.50
Stress–strain index (mm^3)	13,605.4 ± 240.4	13,090 ± 141.6	342.5 ± 23.6	<0.001	0.068	0.66

Table 3. Least square means and standard error for bone parameters in the humerus using periosteal circumference in the metacarpus as a predictor for animals with normal, low and very low liver copper concentration.

Bone Parameter	Normal	Low	Very Low	Coefficient	Metacarpus Periosteal Circumference	*p*-Value Copper	R^2
n	33	31	21				
Bone length (mm)	260.2 ± 1.6 [b]	256.9 ± 1.6 [ab]	251.8 ± 2.2 [a]	1.3 ± 0.2	<0.001	0.013	0.44
Periosteal circumference (mm)	144.3 ± 1.0	142.8 ± 1.0	145.6 ± 1.4	1.3 ± 0.1	<0.001	0.218	0.65
Endosteal circumference (mm)	95.6 ± 1.1	94.5 ± 1.1	97.6 ± 1.6	0.9 ± 0.1	<0.001	0.286	0.42
Total bone area (mm^2)	1663.2 ± 22.7	1627.3 ± 22.3	1694.8 ± 31.7	29.0 ± 2.7	<0.001	0.205	0.65
Total bone content (mg/mm)	1267.2 ± 18.0	1242.1 ± 17.7	1257.8 ± 25.1	20.4 ± 2.1	<0.001	0.607	0.58
Cortical bone thickness (mm)	7.8 ± 0.1	7.7 ± 0.1	7.6 ± 0.2	0.06 ± 0.01	<0.001	0.815	0.19
Cortical bone area (mm^2)	930.6 ± 14.1	913.8 ± 13.8	932.4 ± 19.7	15.1 ± 1.6	<0.001	0.625	0.55
Cortical bone density (mg/cm^3)	1284.7 ± 3.7 [b]	1285.7 ± 3.6 [b]	1270.5 ± 5.2 [a]	−0.4 ± 0.4	0.307	0.045	0.11
Cortical bone content (mg/mm)	1195.4 ± 18.0	1174.1 ± 17.7	1184.4 ± 25.1	19.0 ± 2.1	<0.001	0.702	0.54
Stress–strain index (mm^3)	13,178.5 ± 253.2	12,946.1 ± 248.7	13,463.6 ± 353.7	333.2 ± 29.6	<0.001	0.484	0.65

a, b, c Means with different superscript within row at each age are significantly different ($p < 0.05$).

4. Discussion

Previous research outlined the strong relationship between pQCT measures in the mid-diaphysis of the metacarpus and in the humerus [3]. Therefore, the basis of this work was to identify whether heifers at risk of humeral fractures could be identified using pQCT measures at the mid-diaphysis in the metacarpus. Whilst the mid-diaphysis was shown to not be a useful model for identifying at-risk heifers, the current study provided indications for the temporal pattern of nutritional challenge that is associated with humeral fractures. The general lack of difference in bone morphology in the mid-diaphysis of both the metacarpus and humerus between groups indicates that factors contributing to spontaneous fracture are likely to be recent. Longitudinal bone growth occurs at the metaphysis, making it a dynamic site that can be influenced by recent changes in growth. As longitudinal bone growth occurs, the affected bone produced in the metaphysis is pushed towards the diaphysis. Humeral fractures originate in the metaphysis and thus it is an important site for the quantification of differences in bone parameters. Most longitudinal growth occurs in the first year of a heifer's life, and because of this, changes to bone morphology observed in the diaphysis would occur in the first year. The lack of effect

in the mid-diaphysis, coupled with observations of growth arrest lines in affected bones reported by Dittmer et al. [2], suggest that risk factors causing heifers to be susceptible to humeral fractures must be recent. This result agrees with observations by Handcock, et al. [9], wherein the slowest growth (live weight gain) in a heifer occurs between 20 and 22 months of age. Growth arrest lines occur due to the retardation of longitudinal bone growth, resulting in the temporary sealing of the physis with bone, which is often caused by malnutrition [10]. If the deficit in nutrition is corrected, physeal growth will resume and the seal of the physis will be displaced into the metaphysis, resulting in what is termed a growth arrest line. Therefore, if this proposed timing is correct, the examination of the metaphysis may show reduced cortical bone density and a lower stress–strain index.

Bone size and strength have been shown to be strongly correlated with live weight [6,7]. Due to the opportunistic nature of bone acquisition in both the control and affected groups, live weights could not be consistently or reliably obtained. Therefore, bone parameters could not be adjusted for live weight to give an indication of bone size and strength in relation to animal size. However, previous research has found that bone parameters in the metacarpus can be used as a proxy for humeral bone parameters [3]. The metacarpus undergoes minimal changes after one year of age and provides a gross measure of the size potential of the animal. Therefore, adjusting the humeral bone parameters to a common metacarpus parameter provided a mechanism to adjust for live weight. With the humerus significantly shorter in length in affected heifers, and the tendency for a reduced stress–strain index and cortical bone density, the current results are consistent with observations in human adolescents affected by anorexia nervosa (AN). Severe nutrient deficiency in AN results in low body mass and disruption to the hormones involved in bone regulation [11]. Longitudinal bone growth can be reduced during periods of AN. When sufficient feeding occurs, longitudinal bone growth resumes, but at an accelerated rate. However, the effect on bone mass accrual can be more permanent, resulting in an increased incidence of osteoporosis and fracture risk [12]. The results in the current study and observations of AN emphasize the potential importance of adequate nutrition for optimal bone mass accrual. Bone density will be further reduced at the onset of lactation in dairy cows as a result of calcium being lost from the matrix. Therefore, the onset of lactation is likely to be the tipping point for many heifers that experience spontaneous humeral fractures.

To provide a possible method for the field collection of data, the pQCT voxel size was set at 0.3 mm, which in a field setting provides a suitable trade-off between ability to detect differences and the time of image and data acquisition. A smaller voxel size would have provided greater resolution and reduced the error associated with the voxel sharing effect [13]. However, it was important to test the ability of pQCT-derived values in a setting that would have occurred in the field to accurately test the possibility of using metacarpus values to predict humerus data and risk of fracture. Whilst pQCT provides a tool to quantify bone properties and spatial distribution, it is likely that the resolution used in the pQCT (0.3 mm voxel size) was insufficient to detect subtle changes [14]. Therefore, to detect small changes between the control and affected animals, histology or microCT are required. The current results were limited to the diaphysis due to the limited size of the gantry of the pQCT machine and the large size of the humeral head, and consequently the location of the metaphysis.

Although the relationship between bone parameters and copper was not different between affected and control heifers, low liver copper status was associated with reduced bone length and cortical bone density in the humerus. The reduced cortical bone density indicates that the lack of copper required for collagen cross-linking in bone has reduced the binding of calcium in the matrix. Copper deficiencies also cause a reduction in osteoblastic and osteoclastic activity, resulting in a decrease in osteoid production [15]. This supports the hypothesis of a chronic inadequacy of copper within the diet [16]. Examination of the metaphysis may provide more detail on the effect of recent copper status on bone development, as the metaphysis is the site reported to be most affected by copper deficiencies [15]. Another limitation is the use of copper status at the time of death, as this does

not provide an indication of whether the animal had previously had a copper deficiency and recovered, and if so, how long the animal had been deficient or the severity of the deficit, both of which may impact the bone [5]. The lack of difference in the relationship between bone and copper concentration between affected and control heifers could also mean that copper concentration may not be the primary contributing factor for humeral fractures. The opportunistic nature of the data collection for controls limited the collection of liver copper status data. This low number of liver copper records for controls may have prevented us from identifying the relative influence copper status had in contributing to the risk of humeral fracture.

The comparatively low number of control heifers in this sample reduced the power of the study. With the current number of samples, there was sufficient power to detect a 10% reduction in the stress–strain index in the mid-diaphysis of the humerus. Without correction for body weight, to detect the 0.5% difference observed (Table 1) based on unadjusted means, approximately 29,000 control and affected heifers would be required. In contrast, when adjusting humeral bone parameters for the periosteal circumference of the metacarpus, there was sufficient power to detect a 6% reduction in the stress–strain index. Based on the material collected in this study, there was a 3.7% decrease in stress–strain index, which would have required only 198 control and affected heifers. The difficulty with sampling control heifers at the age of humeral fracture is the limited culling of non-diseased stock at this age. The lack of controls was a limitation, despite significant efforts being made to obtain suitable control samples.

The original aim of this research was to compare bone morphology in the mid-diaphysis of the metacarpus and humerus of both control and heifers affected by spontaneous humeral fractures. If there was a significant difference in morphology between control and affected heifers, future research would aim to establish a field trial to see if the relationship amongst mid-diaphyseal measures in the metacarpus and humerus could be used to identify at-risk heifers in a relatable manner in a field scenario. However, the lack of difference between the metacarpus and humerus in control and affected heifers means the current method will not work. Therefore, further research into the metaphysis of the metacarpus and humerus may provide valuable insight into more recent changes in bone, and allow for the quantification of fracture risk in heifers.

5. Conclusions

Humeral fractures in dairy heifers originate in the distal metaphysis. The mid-diaphysis was a poor predictor of humeral fracture risk in this study. The limited differences in the mid-diaphysis highlighted that the contributing factor for fracture is recent, and may affect bone propagation at the metaphysis, but it had a limited effect on the more mature bone at the mid-diaphyseal site. Temporally, this would relate to the second winter, which has the potential for restricted feed supply due to seasonal pasture growth. Growth arrest lines observed in case–control studies support this hypothesis. Further research should investigate differences in the metaphysis between affected and control heifers, and possibly use a variety of techniques to help elucidate the influence restricted feed supply may have on the development of bone within the metaphysis. The management of dairy heifers in the months prior to calving should also be examined to identify periods of growth restriction that may occur during the second winter or during the transition into the milking herd.

Institutional Review Board Statement: Not applicable.

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