"LOSS" OF HIGH BONE MASS PHENOTYPE IN CFW MICE

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Introduction

• Carworth Farms White (CFW) is a commercially available outbred mouse strain used for bone genetic studies[1,2] ; one study found that some CFW mice have abnormally high aBMD[2].

• ***In the current study, we showed that CFW mice have lost the bimodal distribution of the bone phenotype that was prevalent in previous cohorts.***

Purpose

- To determine the pattern of inheritance for the HBM phenotype (a simple, Mendelian trait or a complex trait).

Previously, we found…

- Of the 103 CFW mice screened (ordered in 3 separate cohorts), 69 had normal bone mass (NBM) and 34 had high bone mass (HBM) phenotype or ***33% of the screened mice had the HBM phenotype, using µCT.***
- Proximal tibia and proximal humerus also had the HBM phenotype, but at a lesser prevalence (Fig. 1).
- The prevalence of the phenotype was evenly distributed among males and females and across the ages studied using radiography (Table 1) and micro-computed tomography (µCT, Table 2).
- Using BV/TV of ≤ 0.45 for the cutoff of normal bone mass and BV/TV ≥ 0.45 for high bone mass (HBM), we found distinct bimodal distributions for BV/TV and vBMD (Fig. 3).

Age (weeks)	Sex	Phenotype	TOTAL
TOTAL	F	18	44
	M	11	35
8	-	7	24
11	-	6	10
16	-	9	30
20-24	-	4	11
28-30	-	3	4

Table 1. Femur samples that were positive (+) for radiographic high bone mass. Total is for all ages combined, showing the sex distribution. For the individual ages, the data were collapsed across sex.

Age (weeks)	Sex	Phenotype	TOTAL
TOTAL	F	19	58
	M	15	45
8	-	5	24
11	-	4	10
16	-	8	30
20-24	-	4	11
28-30	-	10	28

Table 2. Femur samples that were positive (+) for micro-computed-tomography high bone mass. BV/TV: NBM < 0.45, HBM > 0.45 Total is for all ages combined, showing the sex distribution. For the individual ages, the data were collapsed across sex.

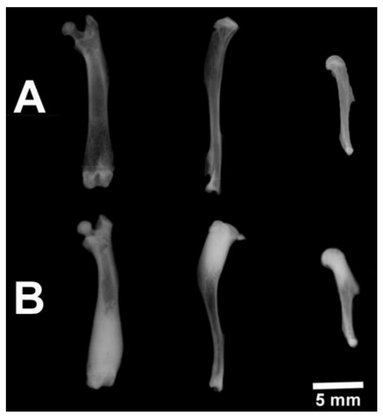

Figure 1. Representative radiographs of femurs, tibiae and humeri from a normal bone density mouse (CFW #13, A) and a high bone density CFW mouse (CFW #17, B). Both mice shown are males, 24 weeks old.

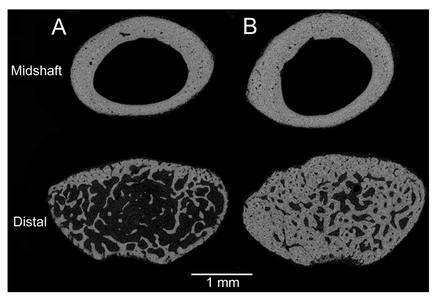

Figure 2. Femur µCT cross sections at midshaft and distal ROIs from a normal bone mass mouse (A) and a high bone mass mouse (B).

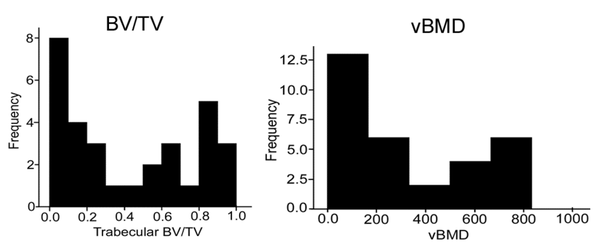

Figure 3. Frequency distributions. Bimodal distal femoral trabecular BV/TV and bimodal vBMD. The population naturally falls into high and normal bone mass groups when the bone is characterized by µCT.

Materials and Methods

- We designed a breeding experiment to assess the pattern of inheritance of the HBM phenotype in the F1 generation of HBM+ X HBM+ and HBM- X HBM- crosses, with the long term goal being to discover the genetics behind phenotypic variance.
- To obtain multiple breeding pairs, 60 CFW mice were ordered (30 females and 30 males; Charles River). At 4, 6 and 8 weeks of age, mice underwent *in vivo* planar radiography (Faxitron) of the distal femur in the presence of calibration phantoms to determine the presence/absence of the HBM phenotype.
- From these *in vivo* radiographs, breeding pairs were established to determine whether HBM phenotype is heritable.
- The breeding pairs were sacrificed at 16 weeks of age. The F1 offspring were sacrificed at 8 weeks of age.
- Right femurs were collected and fixed in 10% neutral buffered formalin and *ex vivo* radiography was performed to determine the presence of HBM or NBM phenotypes.
- Radiographs were analyzed qualitatively and using ImageJ software to determine calibrated grayscale values. From these values, frequency distributions were graphed to determine the incidence of the HBM phenotype.

Parental Generation Radiographic Results

- Of the 60 parental mice screened at 4 weeks of age, only 8 mice (2 females, 6 males) showed a hint of the original HBM phenotype (Table 3). ***13% of the parental generation screened had the 'HBM' phenotype, using in vivo radiographs.***
- These mice were radiographed at 4 (Fig. 4), 6 and 8 weeks (data not shown). The phenotype was not apparent at the later time points.
- From *in vivo* radiographs, frequency distributions were no longer bimodal (Fig. 5).
- *Ex vivo* radiographs, at 16 weeks of age, confirmed the HBM phenotype was not apparent in either sex (Fig. 6).

Age (weeks)	Sex	Possible Phenotype	TOTAL
TOTAL	F	2	30
	M	6	30
4	-	8	60

Table 3. Femur samples that were positive (+) for radiographic high bone mass in parental generation. Total is for all ages combined, showing the sex distribution. For the 4 week age, the data were collapsed across sex.

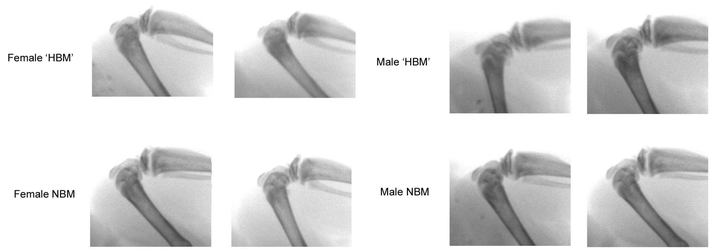

Figure 4. Representative *in vivo* radiographs of parental generation femurs at 4 weeks of age. "HBM'= possible presence of HBM phenotype.

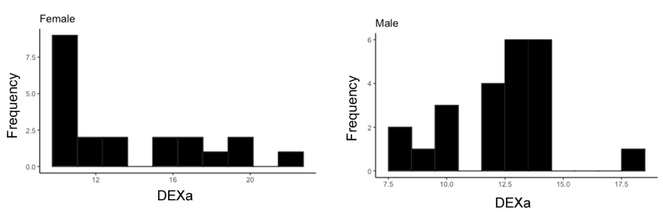

Figure 5. Frequency distributions. Unimodal distal femoral DEXa. The population is no longer distributed into high and normal bone mass groups when the bone is characterized by *in vivo* radiography. 4 weeks of age.

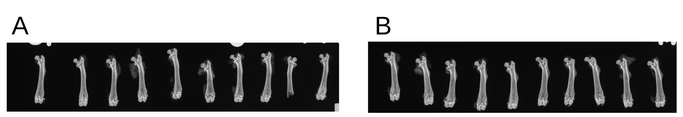

Figure 6. Representative *ex vivo* radiographs of parental generation femurs. At 16 weeks of age, the HBM phenotype is not apparent as in our previously screened cohorts. A- females, B males.

F1 Generation Radiographic Results

- Of the 140 F1 offspring mice screened at 8 weeks of age, NONE showed the HBM phenotype radiographically (Fig. 7).

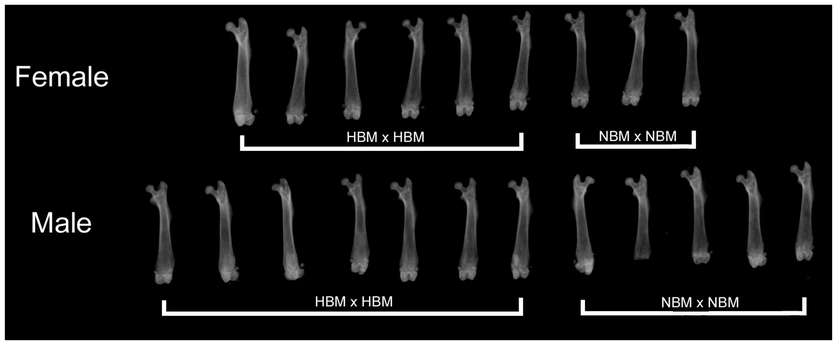

Figure 7. Representative radiographs of F1 offspring femurs at 8 weeks of age. Top row= female F1, bottom row= male F1

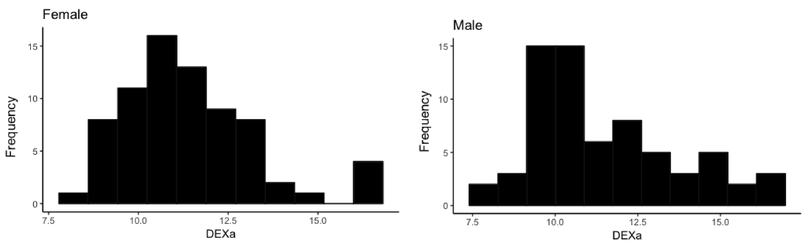

Figure 8. Frequency distributions. Unimodal distal femoral trabecular vBMD. The F1 generation shows a single peak distribution, suggesting the phenotype is no longer present when the bone is characterized by radiography.

Discussion and Conclusion

- Taken together, our studies show that:
- 1) the extreme HBM phenotype once present in >30% of the screened commercially available CFW mice is greatly reduced in the parental generation and in the F1 cohort.
- 2) mouse skeletal phenotypes can be variable despite ordering the same strain from the same vendor. In our case, the breeding room at Charles River changed prior to our breeding experiment, which likely contributed to changes in breeding stock and ultimately, loss of HBM phenotype.
- 3) Due to the loss of HBM phenotype in the CFW mouse strain, we will not be able to meet our goals of 1) determining if the HBM phenotype is a heritable trait and 2) our previous goal of identifying the genetics driving this extreme phenotypic variance.

Acknowledgements

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References

1. Nicod J, Davies RW, Cai N, et al. 2016. Genome-wide association of multiple complex traits in outbred mice by ultra-low-coverage sequencing. Nat Genet 48:912-918. 2. Parker CC, Gopalakrishnan S, Carbonetto P, et al. 2016. Genome-wide association study of behavioral, physiological and gene expression traits in outbred CFW mice. Nat Genet 48:919-926.