THE MOUSE AS A MODELORGANISM FOR HUMAN SKELETAL DISEASES – A BIOMECHANICAL STUDY

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INTRODUCTION

Our understanding of the biology of the skeleton has been transformed dramatically since the first successful introduction expression of selected genes into the germline of mice [1]. Transgenic, knock-out or knock-in strategies – particular through the use of homologous recombination in embryonic stem cells – allow the generation of specific animal models for human diseases to study the regulation and function of genes within mammalian organisms. The power of genetics has also initiated the molecular understanding of the skeleton system and identification of genes responsible for murine and human skeletal abnormalities. Since decades many inbred strains of mice are available, which helped to detect the loci of specific genes responsible, for instance, for the bone mineral density. The biomechanical description of these phenotypes is, although straight, quite challenging due to the size of the analyzed bones. The objective of this study is the integrated biomechanical analysis of various mouse models – inbred and transgenic – representing a broad spectrum of skeletal defects or abnormalities.

METHODS

Five mice-models were selected from the variety of available types: C57BL/6 and FVB/N (age: 12 weeks) as a typical starting model for transgenesis [2,3]; SAM/P6 (age: 12 & 52 weeks) as a model for senile osteoporosis [4]; Calc-$A^{-/-}$ (12 weeks) as a calcitonin knock-out [5]; and $src^{-/-}$ (12 weeks) as an osteopetrosis model [6]. The biomechanical analyses were conducted at the lumbar vertebrae (L4-L6) because they contain a higher amount of trabecular bone than femora. The following methodologies were used: Morphometry: all vertebrae were µCT-scanned (Scanco µ40; resolution: 10µm) and typical parameter like BV/TV, MIL, and trabecular thickness/separation etc. were quantified; Densitometry: bone mineral content (bmc) and density (bmd) were determined experimentally and with the help of a specially designed integrated µCT phantom.; Biomechanics: the failure load and overall stiffness of the vertebrae was determined experimentally with micro-compression test (Figure 1 left) and specimen specific finite element analysis were generated to allow an estimation of yield and ultimate stress at a local tissue-level. These methodologies were combines a the specimen-level to improve the quality of the analysis and inferences

RESULTS AND DISCUSSION

SAM/P6 shows no age-dependent variation in BMD but a distinct loss of BV/TV (from 25 to 17%). Trabecular thickness did not change either but separation increased from 360 to 530µm. Fracture load increased with age although corrected for the size effect of the vertebrae.

FVB has a slightly lower bmd-level than BL6 and failure load is not significantly different when only corrected for the size effect but lower when also corrected for the BV/TV influence – indicating a less strong tissue.

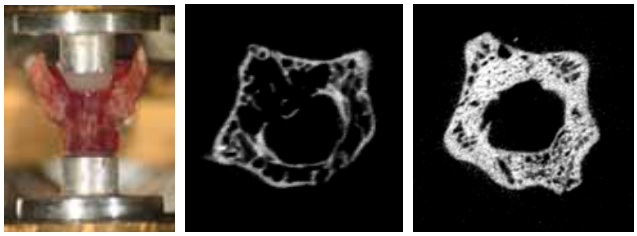

Figure 1: left: micro-mechanical compression test of a vertebra, middle: transverse cross-section of BL6-L4 and right: SRC-L4.

Calc-A has a surprisingly high BV/TV-level compared with the BL6 strain (22% versus 25%) but a reduced trabecular thickness (66µm versus 72µm). The size corrected failure load did not show any significant difference.

The failure load of the $src^{-/-}$ mice is extremely higher than in all other models: 113.7N compared with 32.7 (BL6) but the same is true for the BV/TV-ratio (Figure 1 middle/right): 90% versus 25% (BL6). BMD is also highest of all analyzed mice-models but the difference in the failure load can be largely explained by the size effect and the BV/TV ratio yielding a slightly lower fracture strength in the src-model than in the BL6-strain.

CONCLUSIONS

The analyzed mice-models represent a broad spectrum of skeletal phenotypes and large variations in morphometrical, densitometrical, and biomechanical parameter were measured. The src-model shows the most dramatic variations and indicates the effect of missing osteoclastic activities – yielding a highly mineralized and compact but less strong bony tissue. SAM/P6 shows typical (human) osteoporotic indications like lower BV/TV and higher trabecular separation but lacks reduced compressive strength. Final results of the numerical analyses will additionally give more insight into the tissue strength than current analyses. All vertebrae show a high degree of inhomogeneity and derived morphometric parameters should be treated carefully.

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