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Research Article

Comparison of gene expression of bone regulators and bone microstructure in 20 weeks old female mice of two different strains (C57BL/6J and C3H/HeOuJ)

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Abstract

A huge number of inbred mouse strains with different bone properties have become available for musculoskeletal research. C57Bl/6J and C3H/HeOuJ mice show a significant difference in their bone characteristics. Nevertheless, there is a lack of knowledge on the molecular basis of these strain differences. The aim of this study is to determine the gene expression of selected regulators expressed in the bone marrow as well as bone microstructure of C57Bl/6J and C3H/HeOuJ mice. Bone properties were investigated in 20-week-old female C57Bl/6J and C3H/HeOuJ mice. Total RNA was extracted from the bone marrow of the tibia and gene expression of the following genes was determined by quantitative real-time PCR: SOST, DKK1, OPN, FGF23, RANKL, IL6, TNF, IL17a, and OPG. The femur and third lumbar vertebral body (L3) were investigated by μCT. Bone histomorphometric evaluations were performed in tartrate-resistant acid phosphatase/toluidine blue stained fourth lumbar vertebral bodies (L4). C57Bl/6J and C3H/HeOuJ mice showed significant differences in the gene expression of DKK1, FGF23, IL-6, TNF, and OPG. When compared with C57Bl/6J mice, C3H/HeOuJ mice had a stronger cortical and trabecular bone microstructure at the femur. In contrast, at L3 bone volume/total volume (BV/TV) and trabecular number were significantly higher in C57Bl/6J than in C3H/HeOuJ mice. Bone histomorphometry of L4 revealed significantly higher BV/TV, trabecular number, and thickness in C57Bl/6J mice. Furthermore, the number of osteoblasts and the number of osteoclasts/bone perimeter were higher in the C57Bl/6J mice. This study shows that C57Bl/6J and C3H/HeOuJ mice exhibit a differential expression of cytokines present in the bone marrow. Bone properties differ not only between both strains but also in relation to the investigated bone region.

Keywords: bone metabolism, RNA, mouse strains, bone histomorphometry, microCT, osteoimmunology

Lay Summary

Over the last decades, a huge number of different mouse strains have become available for bone research. C57Bl/6J and C3H/HeOuJ mice are two well-known examples of such strains. Until the present day, it is not known how they differ on the molecular basis. Due to this, we investigated the gene expression of selected regulators, which are responsible for bone formation and/or resorption. For this, we extracted bone marrow from the tibia of female C57Bl/6J and C3H/HeOuJ mice and isolated RNA to see how the different regulators vary between these mice. Additionally, we performed microCT and bone histomorphometry of the femur and vertebral bodies to see how their bone properties relate with the gene expression. With our investigations, we saw significant differences in the gene expression of the bone regulators between both strains. Moreover, we saw that the bone properties differ not only between the strains but also in relation to the investigated bone region.

Introduction

Over the last years, a lot of different inbred mouse strains have become available for biomedical research and gained importance in the field of bone research. It is crucial to know that different mouse strains differ in regards to bone mass and biomechanical bone properties.[1] C57Bl/6J and C3H/HeOuJ mice are two examples, which show a significant difference in their BMD. Overall, C57Bl/6J exhibit a low bone mass phenotype, whereas C3H/HeOuJ mice are characterized by high BMD.[2,3] Comparing the femora of C57Bl/6J and C3H/HeOuJ mice, it is described that C57Bl/6J mice develop stiffer, stronger, and tougher bones with aging, whereas aging C3H/HeOuJ mice also showed stiffer but more brittle femora.[4] Looking at the femur density, the C3H/HeOuJ strain has 53% denser femur than that in the C57Bl/6J mice.[5]

Mice reach peak bone density at around 16-24 weeks of age, which can vary between different mouse strains. The lower the peak bone density, the higher are the chances to develop osteopenia. Comparing C57Bl/6J and C3H/HeOuJ mice, C3H/HeOuJ mice are described to have a higher peak bone density.[5] Moreover, C3H/HeOuJ reach their maximal

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skeletal biomechanical properties before 16 wk of age, which is not applicable for C57Bl/6J mice.[4] Comparing serum osteocalcin (OC) levels, which is a marker for bone formation, C57Bl/6J mice showed higher values. Serum alkaline phosphatase (ALP), another marker for osteoblast activity, is significantly higher in C3H/HeOuJ mice.[6] In general, it is described that C3H/HeOuJ mice have a greater bone formation and lower bone resorption, in comparison with the C57Bl/6J strain.[6,7] The higher bone density present in C3H/HeOuJ mice is due to a higher bone formation rate, which is associated with an increased osteoblast activity and lower apoptosis levels.[8]

C57Bl/6J and C3H/HeOuJ mice also differ in relation to intestinal calcium absorption and activity of calcium-regulating hormones, as the absorption is described to be higher in C3H/HeOuJ mice during the period of rapid bone acquisition. Moreover, C3H/HeOuJ mice absorb more calcium than the C57Bl/6J strain.[2]

Looking at mechanical loading, exercises lead to stronger bones in C57Bl/6J mice, but not in C3H/HeOuJ.[9] Immobilization or disuse of the limb lead to a higher amount of bone loss in C57Bl/6J mice in comparison with C3H/HeOuJ mice.[10] Furthermore, it leads to greater cortical and trabecular bone loss in C57Bl/6J compared with C3H/HeOuJ mice.[11]

Based on our current knowledge, we hypothesized that the bone parameters of C57Bl/6J and C3H/HeOuJ mice not only differ in terms of the strains but also in relation to the bone region examined. We assumed that in mice the expression of regulators of bone metabolism is strain dependent. Specifically, we expected that in the mouse strain with advantageous bone properties, regulators that favor osteoblasts are higher and those that inhibit osteoclasts are lower than in a strain with inferior bone properties. The aim of this study was to determine the gene expression of selected regulators expressed in the bone marrow of female C57Bl/6J and C3H/HeOuJ mice. Furthermore, we evaluated the trabecular and cortical bone microstructure in different bone regions in more detail as well as the levels of bone turnover markers.

Materials and methods

Animals

Ten female C57Bl/6J and ten C3H/HeOuJ mice were obtained from Charles River, Sulzfeld, Germany and "Abteilung für Labortierkunde und Genetik", Zentrum für Biomedizinische Forschung, Medizinische Universität Wien (MUW) at the age of 10 weeks. At our animal facility (Department of Pathophysiology and Allergy Research, MUW), animals were maintained in groups of 2-6 mice per cage under a standard 12h light-dark cycle and had unlimited access to drinking water and food (ssniff R/M-H autoklavierbar, ssniff Spezialdiäten GmbH). At the age of 20 weeks mice were euthanized by carbon dioxide asphyxiation and afterwards the femur, tibia, vertebral bodies (L3 and L4), and spleen were collected. One mouse per group was used for additional preliminary experiments that did not work out properly, therefore all the mentioned experiments, accept ELISA, were performed only with nine mice per group. All procedures were performed in accordance with the national and institutional laws and regulations.

RNA isolation, reverse transcription, and quantitative real-time RT-PCR

Total RNA was extracted with peqGOLD Trifast (VWR) from the bone marrow isolated from the tibia using ReliaPrep RNA Tissue Miniprep System (Promega GmbH) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA using High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) and used for quantitative PCR. Real time RT-PCR was performed for *Sost* (Mm00470479_m1), *Dkk1* (Mm00438422_m1), *Spp1/Opn* (Mm00436767_m1), *Fgf23* (Mm01183126_m1), *Tnfsf11/RANKL* (Mm00441906_m1), *Il6* (Mm00446190_m1), *Tnf* (Mm00443258_m1), *Il17a* (Mm00439618_m1), and *Tnfrsf11b/OPG* (Mm00435454_m1) using TaqMan Gene Expression Assay (Invitrogen, Thermo Fisher Scientific) following the manufacturer's instructions. The results were normalized to an invariant endogenous control (*Gapdh*, Mm99999915_g1) and calculated applying the ΔΔCT method.[12] They are presented as fold increase relative to *Gapdh* expression. RNA from a single mouse spleen was isolated and used as a calibrator.

Bone microCT

The trabecular and cortical compartments of the femur and 3rd lumbar vertebral body (L3) were assessed by micro-computed tomography (microCT) (μCT35, Scanco Medical) according to the American Society for Bone and Mineral Research (ASBMR) guidelines.[13] The measurements and evaluations were performed as described previously.[14,15]

Paraffin embedding

For paraffin embedding, the formaldehyde-fixated 4th lumbar vertebral body (L4) was decalcified with trisamino methane-ethylendiaminetetraacetic acid overnight at room temperature (RT) and afterwards dehydrated with increasing alcohol concentrations (70, 80, 96, and 100%) at RT for 1 hr each. Before embedding, samples were transferred to xylol for 30 min at RT. Afterwards, the samples were infiltrated with paraffin and embedded as described previously.[16] Sectioning was performed with the Microtome Microm HM355S (Thermo Fisher Scientific), using specific knives for hard materials like bone.

Tartrate-resistant acid phosphatase/toluidine blue staining

To visualize the present osteoclasts, osteoblasts, cortical, and trabecular structures of the L4, tartrate-resistant acid phosphatase (TRAP) (Sigma-Adrich/Serva)/toluidine blue (Fluka Analytical, Sigma-Aldrich) staining was performed on paraffin sections according to the manufacturer's protocol. For toluidine blue counterstaining, 1% toluidine blue solution (pH 4.5) was 1:50 diluted and applied for 2 min.

Bone histomorphometry

Histomorphometric analysis was performed using the Osteomeasure™ system (OsteoMetrics). The standardized nomenclature of the ASBMR Histomorphometry Nomenclature Committee was applied.[17] For determination of trabecular, osteoblastic, and osteoclastic parameters the above-mentioned TRAP/toluidine blue stained L4 were used and manually evaluated. For the evaluation of the trabecular bone in lumbar vertebrae of mice, it is recommended to use 250 μm distance from the cranial and caudal growth plate. Cancellous bone within 250 μm from the endocortical bone surface should be excluded to be sure that no endocortical bone remodeling activity is included.[18] Due to these recommendations, we set the measuring area of the trabecular compartment at 250 μm from the intervertebral

disc. As cutting bone is tricky due to brittleness, we were only able to analyze seven L4 by bone histomorphometry.

Enzyme-linked immunosorbent assay (ELISA)

Blood samples were obtained by cardiac puncture immediately after euthanasia and centrifuged at 2500 × *g* for 10 min and stored at −70 °C until analysis for bone turnover markers. The C-terminal telopeptide of type I procollagen (CTX; RatLaps, Immunodiagnostic Systems IDS), a marker of bone resorption and procollagen type 1 ami-no-terminal propeptide (P1NP; RatLaps, Immunodiagnostic Systems IDS) a marker indicative of bone formation, were assessed with ELISAs according to the manufacturer's protocol.

Statistical evaluation

Statistical evaluation was performed using GraphPad Prism (GraphPad Software). Unless stated differentially, data are either presented as box plots (median, quartiles, maximum, and minimum) or dot plots (including the mean values). For statistical evaluations, the data were checked for normal distribution and either a Student *t*-test or Mann–Whitney U-test was used. *p*-values <.05 were considered as statistically significant.

Results

Body weight of mice

The measured body weights of both strains showed a significant difference between C57Bl/6J and C3H/HeOuJ mice (24.2 ± 1.2 g vs 35.9 ± 2.2 g, $^{***}p < .0001$). The data is shown in the supplementary material (Figure S1).

Bone microCT of 3rd lumbar vertebral body (L3)

Figure 1 shows the 3D reconstruction of the μCT measurement of one L3 performed in both mouse strains. Furthermore, it shows the trabecular bone microstructure of L3 measured by μCT. We saw significant differences in all measured parameters between both strains. C57Bl/6J mice revealed a higher trabecular number, with lower thickness and separation. Moreover, C57Bl/6J mice showed higher BV and BV/TV values, compared with the C3H/HeOuJ mice. Looking at the bone surface and specific bone surface, C57Bl/6J mice had significantly higher values compared with C3H/HeOuJ mice (Figure S2).

TRAP/toluidine blue staining and bone histomorphometry

Figures 2 and 3 show TRAP/toluidine blue staining of L4 and the bone histomorphometric evaluation investigated in the L4 of both mouse strains. In the trabecular parameters we saw significantly higher values in BV/TV, trabecular thickness, and number in the C57Bl/6J mice. Moreover, C57Bl/6J mice showed significantly higher numbers of osteoclasts/bone perimeter and had a higher osteoclast surface. Looking at the bone area, C57Bl/6J mice showed significantly higher values.

Bone microCT of femur

Figures 4 and 5 show the 3D reconstruction of the μCT measurement of one femur performed in both mouse strains and the strain related differences in the trabecular and cortical bone microstructure measured in the femur by μCT. Looking at the cortical parameters, C3H/HeOuJ mice showed significantly higher values. The evaluation of the trabecular bone microstructure showed a significantly higher trabecular number and thickness and a lower separation in the C3H/HeOuJ strain. Furthermore, C3H/HeOuJ mice showed a significantly higher BV and BV/TV, in comparison with C57Bl/6J mice. The bone surface was significantly higher in C3H/HeOuJ mice compared with C57Bl/6J mice. In contrast to that, specific bone surface was significantly higher in C57Bl/6J mice (Figure S3).

Real-time PCR

Figure 6 shows the gene expression measured by RT-PCR from the bone marrow isolated from the tibia of C57Bl/6J and C3H/HeOuJ female mice. We could obtain significant differences in the expression of DKK1, FGF23, IL-6, TNF, and OPG between both mouse strains. C3H/HeOuJ mice had significantly higher DKK1, IL-6, TNF, and OPG expressions, whereas C57Bl/6J showed higher FGF23 expression in the bone marrow.

Enzyme-linked immunosorbent assay (ELISA)

The performed CTX and P1NP ELISA showed no significant difference between both strains (CTX: 14.7 ± 2.4 ng/mL vs 13.0 ± 2.0 ng/mL and P1NP: 2.2 ± 0.3 ng/mL vs 2.1 ± 0.3 ng/mL). C57Bl/6J mice showed slightly higher CTX values, which is in line with the higher osteoclast number detected by bone histomorphometry. The data are shown in the supplementary material (Figure S4).

Discussion

With the aim to investigate further strain specific differences between C57Bl/6J and C3H/HeOuJ mice, especially at the molecular level, we performed quantitative real-time PCR to determine the gene expression of selected bone regulators expressed in the bone marrow of female C57Bl/6J and C3H/HeOuJ mice. Furthermore, we evaluated trabecular and cortical bone characteristics in different bone regions in more detail by μCT and bone histomorphometry. The bone turnover markers CTX and P1NP of these two strains were evaluated by ELISA.

With regard to the gene expression evaluated from the bone marrow, we expected to see in C3H/HeOuJ mice, with advantageous bone properties, higher regulators that favor osteoblasts and lower regulators that inhibit osteoclasts in comparison with a mouse strain with inferior bone properties, like C57Bl/6J. Our investigations showed unexpected results as IL-6 and TNF, known osteoclastogenic cytokines, were higher in C3H/HeOuJ mice. Moreover, DKK1 expression, a bone formation inhibitor, was also higher expressed in C3H/HeOuJ mice. In comparison with that, the increased OPG expression in C3H/HeOuJ mice is consistent with our hypothesis. FGF23, which is important for osteoblast differentiation, was higher expressed in C57Bl/6J mice (see Figure 6). Comparing our results with previous publications, Ganapamo et al.[19] described that after an infection with *Borrelia burgdorferi* C3H/HeOuJ mice produced significant more IL-6, compared with C57Bl/6J. Similar results were presented by Gautam et al.,[20] where C3H/HeN mice showed higher IL-6 and TNFα levels after an infection with *B. burgdorferi*, compared with C57Bl/6J mice. Zhang et al.[21] described the

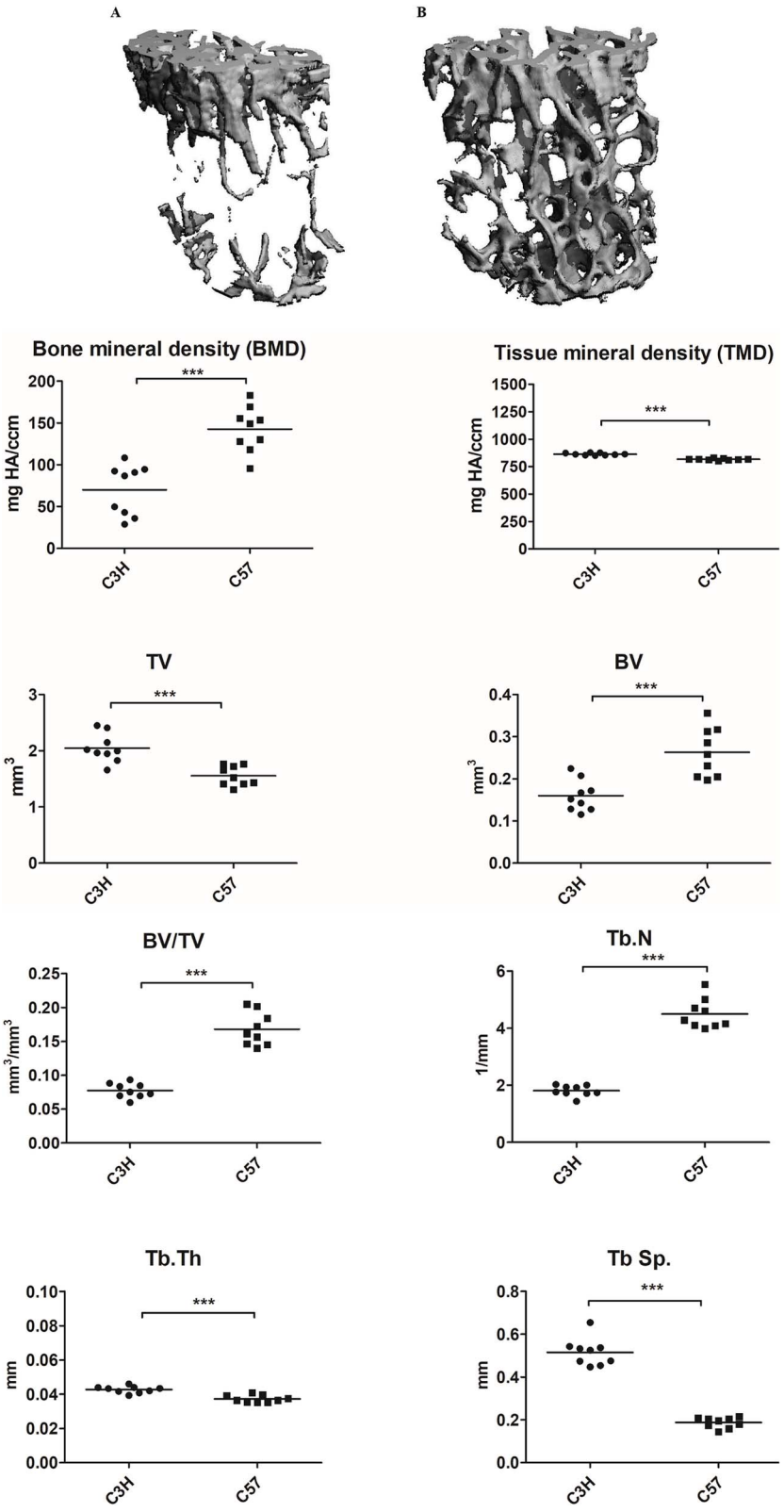

Figure 1. 3D reconstruction of the μCT measurement of one L3: (A) C3H/HeOuJ and (B) C57Bl/6 mouse and μCT measurement of L3:tissue mineral density (TMD) $^{***}p < .0001$, BMD $^{***}p < .0001$, total volume (TV) $^{***}p = .0002$, bone volume (BV) $^{***}p = .0003$, bone volume/total volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb Sp.) $^{***}p < .0001$ ($n = 9$ per group).

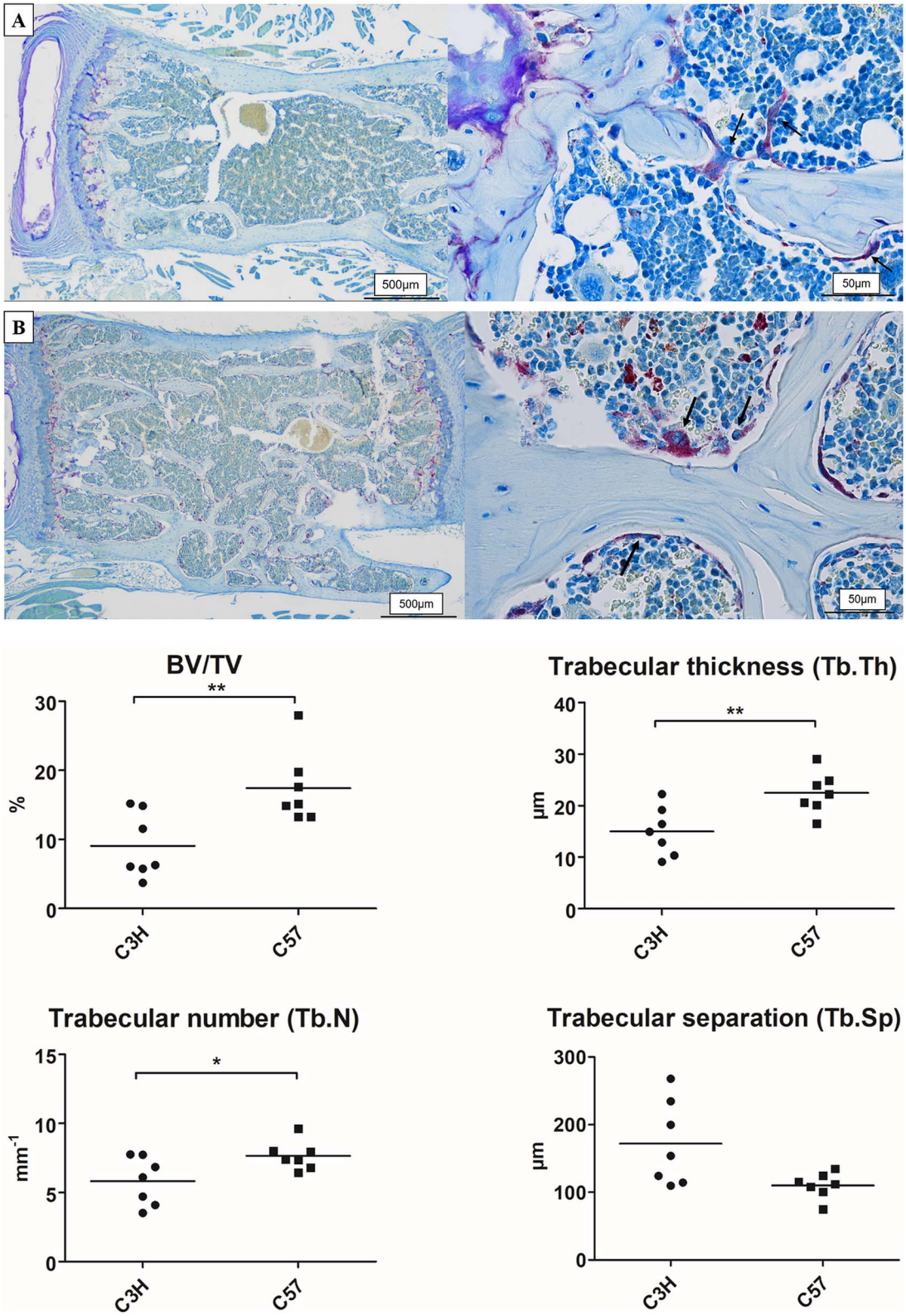

Figure 2. TRAP/toluidine blue staining of L4 of (A) C3H/HeOuJ mice and (B) C57Bl/6J mice. The arrows indicate multinucleated osteoclasts. Original magnification 40× and 400× and bone histomorphometry L4: bone volume/total volume (BV/TV) $^{**}p = .0085$, trabecular thickness (Tb.Th) $^{**}p = .0075$, trabecular number (Tb.N) $^{*}p = .0335$ and trabecular separation (Tb.Sp) ($n = 7$ per group).

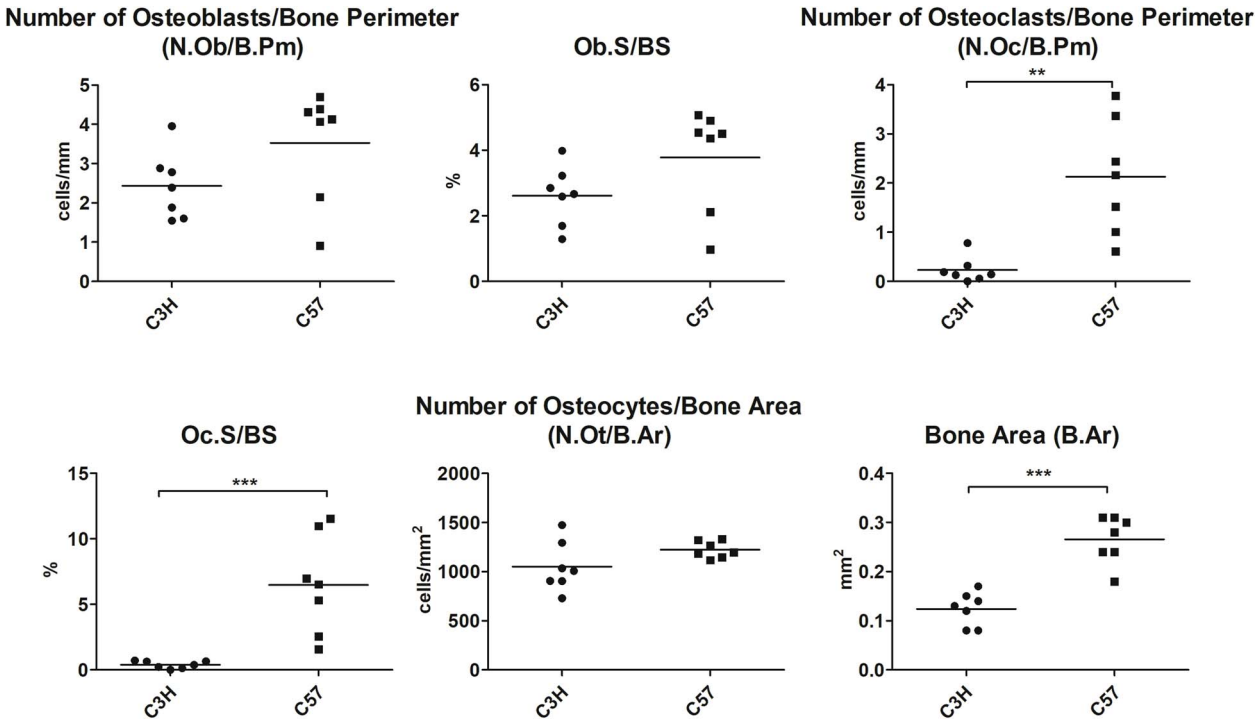

Figure 3. Bone histomorphometry L4: number of osteoblasts/bone perimeter (N.Ob.B.Pm), osteoblast surface (Ob.S/BS), number of osteoclasts/bone perimeter (N.Oc/B.Pm) $^{**}p = .0012$, osteoclast surface (Oc.S/BS) $^{***}p < .0006$, number of osteocytes/bone area (N.Ot/B.Ar) and bone area (B.Ar) $^{***}p < .0001$ ($n = 7$ per group).

plasma cytokine levels in C57Bl/6 and C3H/HeN mice, showing higher IL-6 and TNFα levels in C3H/HeN mice, similar to our findings. Matsutani et al.[22] compared the cytokine production in two different mouse strains (C57BL/6 and C3H/HeN) and showed that C3H/HeN mice had a higher TNFα expression, similar to our findings. A possible explanation of how higher TNFα expression may be associated with stronger bone properties is presented by Balga et al.,[23] who described that at relatively low concentrations, this cytokine may exert an inhibitory effect on osteoclastogenesis.

Although the bone turnover markers did not show any significant differences, a trend toward higher CTX values in the C57Bl/6J strain was detectable (see Figure S4). This is consistent with the higher number of osteoclasts present in the L4. Contrasting the findings of the bone turnover markers and bone histomorphometry (see Figures 2 and 3) the latter method was far more sensitive revealing differences in the two investigated strains. Kim et al.[24] investigated CTX and P1NP serum values in C57/BL6 and C3H/HeNHsd mice and revealed different results, as both parameters were higher in C3H mice. In the femur, however, we hypothesize that C57Bl/6J mice may have lower osteoblast numbers, as DKK1 expression measured from the bone marrow of the tibia is higher in C3H/HeOuJ mice. The tibia and femur are both long bones, and therefore we assume similar bone properties. The low osteocyte number present in the L4 is consistent with the higher expression of DKK1 in C3H/HeOuJ mice, as DKK1 inhibits osteoblasts from which osteocytes develop. The higher OPG expression detected in the same mice is compatible with the bone histomorphometric evidence of lower bone turnover in the C3H/HeOuJ strain. Akhter et al.[25] described the bone histomorphometric differences found in L5 of C3H/HeJ and C57BL/6J. Similarities to our findings included a higher BV/TV and trabecular number in C57BL/6J. Trabecular separation was higher in C3H/HeJ mice. In contrast to our findings, trabecular thickness was higher in C3H/HeJ. Looking at the biomechanical strength properties, no significant differences between the two strains were detected.

Our μCT findings (see Figures 1, 4, and 5) were consistent with previously published studies. Papageorgiou et al.[15] described that the L4 in C57Bl/6J mice had higher BV/TV, trabecular number, and spacing, which is comparable with our findings. Only the trabecular thickness differed from our results. Looking at the femur, C3H/J mice showed higher cortical and trabecular thickness, similar to our findings. BV/TV values revealed similarities between 16-week-old C3H mice and our described results. The tibia of C3H/J mice had higher cortical and trabecular thickness values. Föger-Samwald et al.[26] showed that trabecular parameters, such as BV/TV, trabecular number, and thickness were higher in the femur of C3H/HeOuJ mice, which is consistent with our results. Furthermore they showed a higher cortical thickness but a smaller trabecular distance, in comparison with the C57Bl/6J strain. Bouxsein et al.[27] described higher BA/TA in the femur and higher BV/TV and trabecular thickness in the tibia of C3H mice. Looking at mechanical strength, Kodama et al.[9] described that the femur of C57Bl/6J mice breaks with less forces, compared with C3H mice. In the study of Turner et al.[3] similar results were described. The L5 exhibited higher BV/TV and trabecular number in C57Bl/6J mice. In contrast to that, C3H/HeOuJ mice had a higher trabecular density and spacing, which is similar to our results. Buie et al.[28] showed similar results, as the BV/TV and trabecular number were higher and trabecular thickness and separation lower, in L3 in C57Bl/6J mice compared with C3H/HeN mice. The tibia

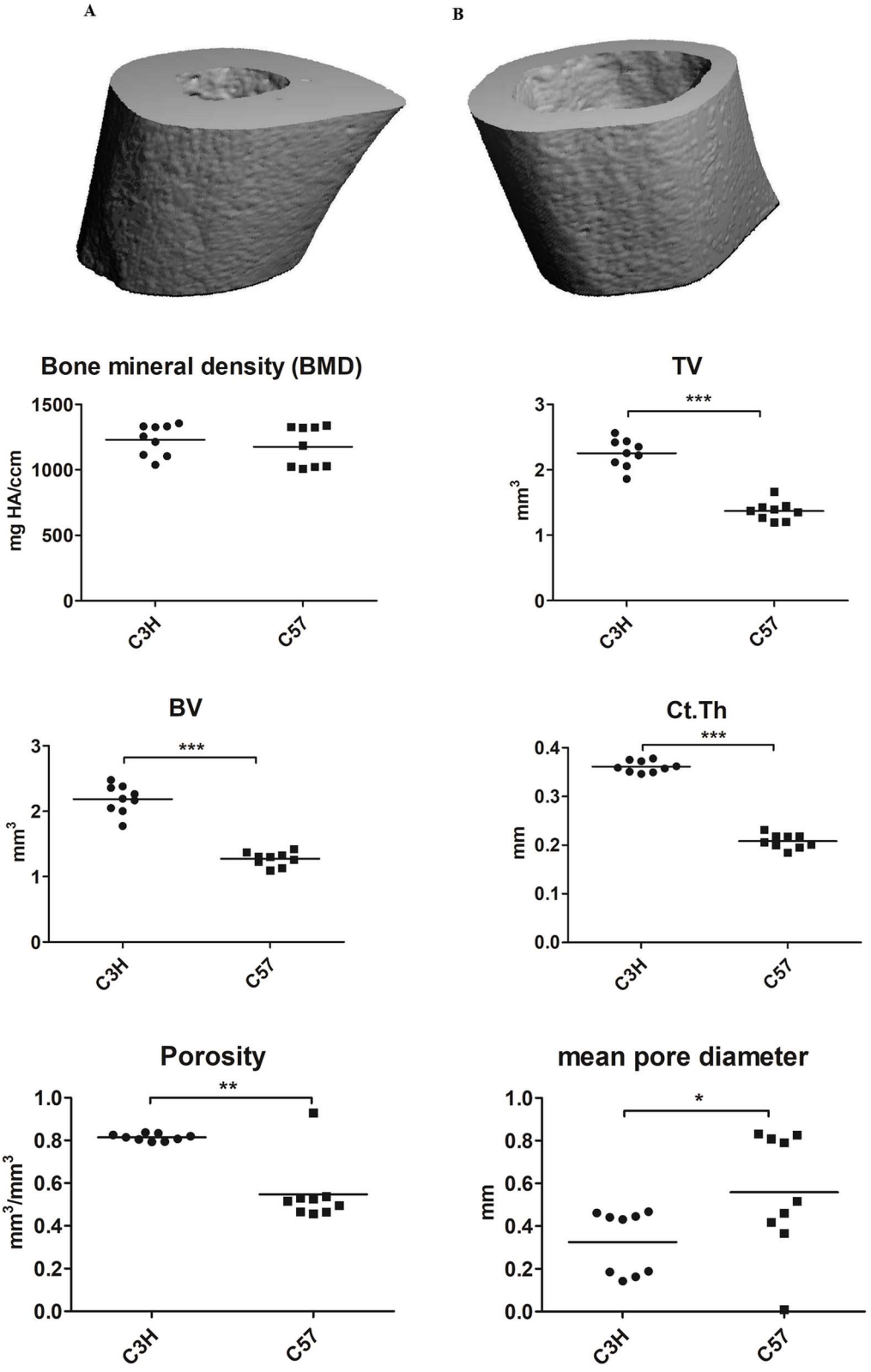

Figure 4. 3D reconstruction of the μCT measurement of one femur (A) C3H/HeOuJ and (B) C57Bl/6 mouse and μCT measurement of femur: BMD, total volume (TV) ***$p < .0001$, bone volume (BV) ***$p < .0001$, cortical thickness (Ct.Th) ***$p < .0001$, porosity **$p = .0040$ and mean pore diameter *$p = .0424$ ($n = 9$ per group).

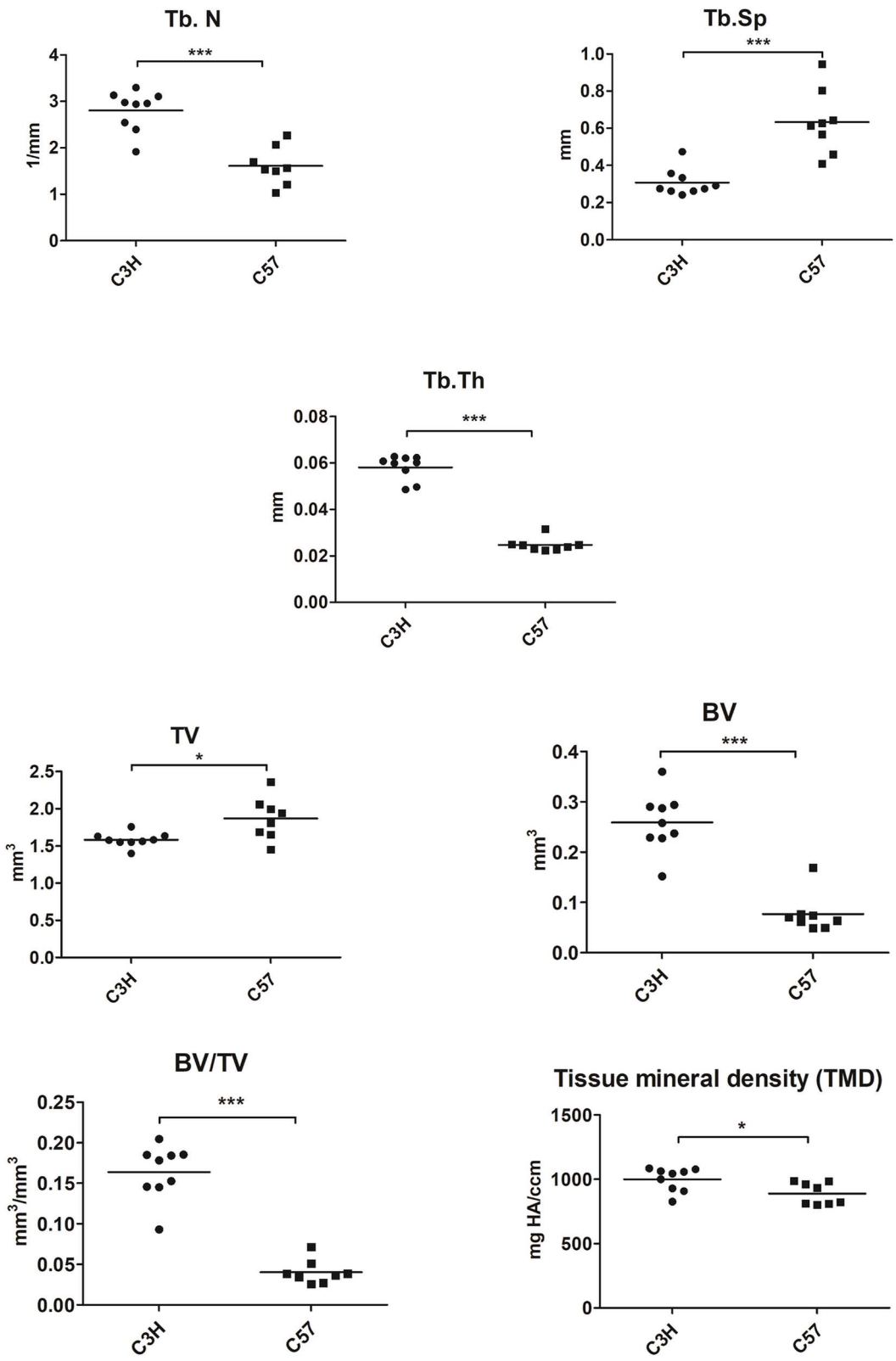

Figure 5. μCT measurement of femur: trabecular number (Tb.N) ***$p < .0001$, trabecular separation (Tb.Sp) ***$p = .0001$, trabecular thickness (Tb.Th) ***$p < .0001$, TV *$p = .0109$, bone volume (BV) ***$p = .0002$, bone volume/total volume (BV/TV) ***$p < .0001$ and tissue mineral density (TMD) *$p = .0213$ ($n = 9$ per group).

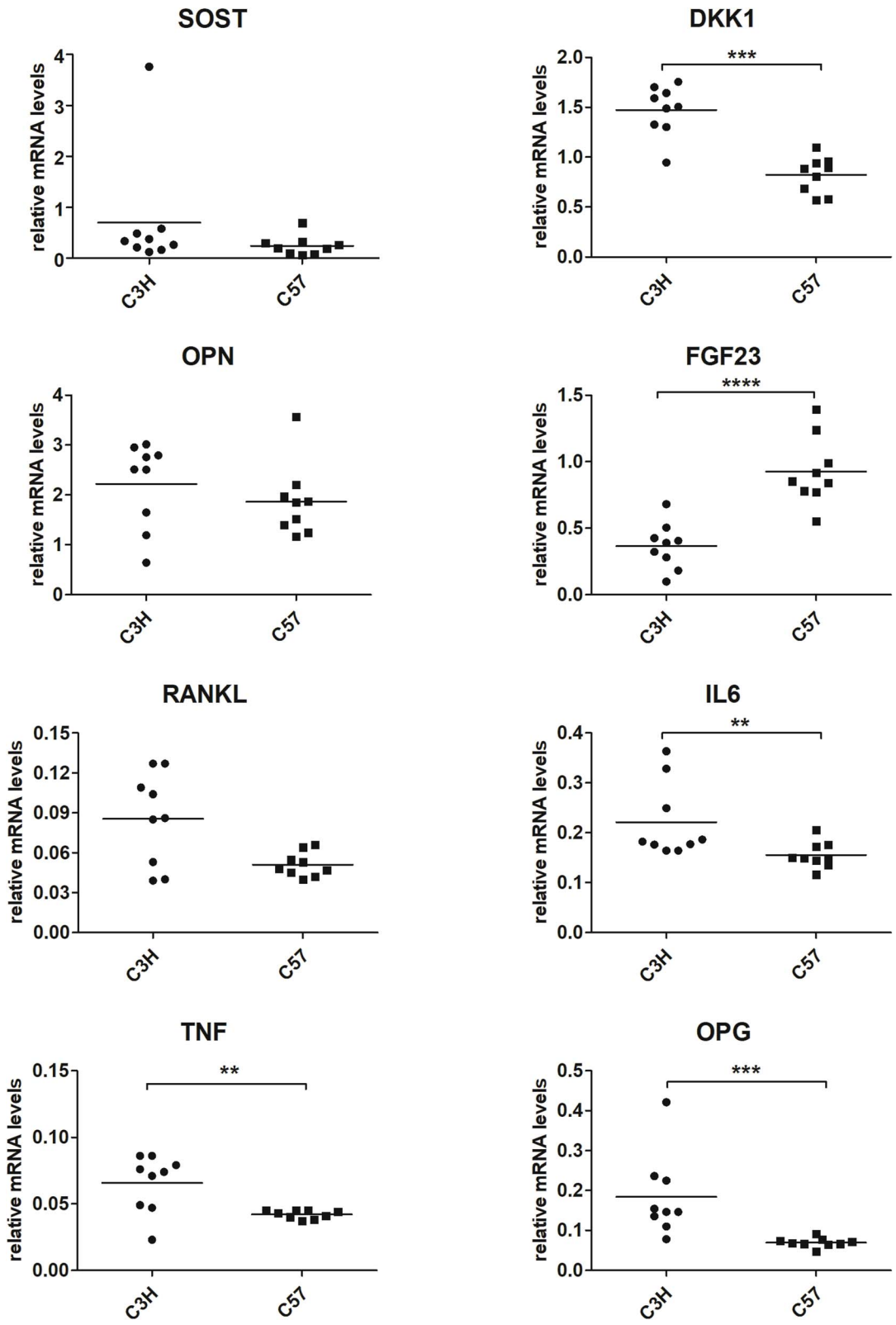

Figure 6. Dot plots of gene expression of Sclerostin (SOST), Dickkopf-1 (DKK1) ***$p = .0002$, Osteopontin (OPN), fibroblast growth factor (FGF23) ****$p < .0001$, RANK ligand (RANKL), Interleukin-6 (IL6) **$p = .0062$, TNF **$p = .0037$ and Osteoprotegerin (OPG) ***$p = .0006$. RNA was extracted from bone marrow isolated from the tibia ($n = 9$ per group).

of C3H/HeN mice had higher BV/TV, cortical and trabecular thickness, and trabecular number, compared with C57Bl/6J mice. Only trabecular separation was higher in C57Bl/6J mice. Analyzing the μCT results of L3 in contrast to the femur, we saw surprising differences between both bone regions. We expected to see in both regions higher BV densities in C3H/HeOuJ mice, but in L3 this was not the case. Nevertheless, specific bone surface (BS/BV) was significantly higher in C57Bl/6J mice at both investigated sites, indicating a generally higher structural complexity in comparison with C3H/HeOuJ mice (see Figures S2 and S3). Looking at the tibia and L3 in both strains, Buie et al.[28] had similarities with our results in the C57Bl/6J and C3H strains. BV/TV and trabecular thickness was higher in L3 and trabecular separation in the femur of C57Bl/6J mice. C3H mice only showed similarities in the BV/TV measured in the femur. A possible reason for these unexpected differences between L3 and femur might be mechanical loading, as the loading pattern between the vertebral column and long bones can be different in rodents. It was described previously that there are significant differences in bone formation responses to mechanical loading between the two mouse strains with C3H/HeJ showing a very low response to mechanical loading.[9,29] Furthermore, C57Bl/6J mice seem to have a higher cage activity, which might impose a higher mechanical stress on their vertebral column.[30,31] Another reason might be the lower number of osteoblasts present in C3H/HeOuJ mice, which could be associated with the low response to mechanical loading. It is described that mechanical unloading leads to less responsiveness of osteoprogenitors and osteoblasts to IGF-1 and PTH.[30,32,33]

Comparing our μCT and bone histomorphometric evaluations, we saw between L3 and L4 similarities in all parameters, except trabecular thickness. Nevertheless, it should be taken into consideration that μCT is a 3D evaluation tool, whereas bone histomorphometry only is a 2-dimensional evaluation of tissue. We therefore consider the 3D evaluation of trabecular structures by μCT as more robust than the 2D evaluation by histomorphometry.

In summary, bone properties differ not only between both strains but also in relation to the investigated bone region.

Conclusion

This study shows that C57Bl/6J and C3H/HeOuJ mice exhibit a differential expression of cytokines present in the bone marrow. This is partly consistent with the known differences present in the bone properties of these two strains. A limitation of this study might be missing protein analysis from the bone marrow, which would give us a broader insight in the differences on the protein level in both strains. Another limitation we want to mention is the fact that we only investigated female mice with the same age. It is known that female and male and young and old mice differ in their bone properties. Moreover, it would have been interesting to perform μCT not only on L3 and the femur but also at the tibia. Nevertheless, our results expand the knowledge of different mouse strains and are highly relevant for the design of future preclinical studies. Looking at the gene expression levels found in the bone marrow of both strains, cytokine expression was higher in C3H/HeOuJ and FGF23 expression higher in C57Bl/6J mice. These novel findings can influence the choice of the specific mouse strain in future studies, depending on the study aim.

Author contributions

Maria Butylina (Investigation, Methodology, Visualization, Writing—original draft, Writing—review & editing), Marie-Christine Priklopil (Investigation, Methodology, Visualization, Writing—review & editing), Michael Hudec (Investigation, Methodology, Writing—review & editing), Katharina Wahl-Figlash (Investigation, Methodology, Writing—review & editing), Katharina Gelles (Investigation, Methodology, Writing—review & editing), Janina Patsch (Conceptualization, Funding acquisition, Resources, Writing—review & editing), and Peter Pietschmann (Conceptualization, Funding acquisition, Supervision, Writing—original draft, Writing—review & editing).

Supplementary material

Supplementary material is available at *JBMR Plus* online.

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Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data that support the findings of this study are available on request by the corresponding author.

References

1. Rosen CJ, Beamer WG *Mouse Genetics as a Tool to Study Bone Development and Physiology;* Elsevier, 2010, https://doi.org/10.1016/B978-0-12-375098-3.50012-3.
2. Chen C, Kalu DN. Strain differences in bone density and calcium metabolism between C3H/HEJ and C57BL/6J mice. *Bone*. 1999;25(4):413–420. https://doi.org/10.1016/S8756-3282(99)00185-4
3. Turner CH, Hsieh Y-F, Müller R, et al. Genetic regulation of cortical and trabecular bone strength and microstructure in inbred strains of mice. *J Bone Miner Res*. 2000;15(6):1126–1131. https://doi.org/10.1359/jbmr.2000.15.6.1126
4. Voide R, van Lenthe GH, Müller R. Differential effects of bone structural and material properties on bone competence in C57BL/6 and C3H/He inbred strains of mice. *Calcif Tissue Int*. 2008;83(1):61–69. https://doi.org/10.1007/s00223-008-9120-y
5. Beamer WG, Donahue LR, Rosen CJ, Baylink DJ. Genetic variability in adult bone density among inbred strains of mice. *Bone*. 1996;18(5):397–403. https://doi.org/10.1016/8756-3282(96)00047-6
6. Richman C, Kutilek S, Miyakoshi N, et al. Postnatal and pubertal skeletal changes contribute predominantly to the differences in peak bone density between C3H/HeJ and C57BL/6J mice. *J Bone Miner Res*. 2001;16(2):386–397. https://doi.org/10.1359/jbmr.2001.16.2.386
7. Akhter MP, Iwaniec UT, Covey MA, Cullen DM, Kimmel DB, Recker RR. Genetic variations in bone density, histomorphometry, and strength in mice. *Calcif Tissue Int*. 2000;67(4):337–344. https://doi.org/10.1007/s002230001144
8. Sheng MH-C, Baylink DJ, Beamer WG, et al. Histomorphometric studies show that bone formation and bone mineral apposition rates are greater in C3H/HeJ (high-density) than C57BL/6J (low-density) mice during growth. *Bone*. 1999;25(4):421–429. https://doi.org/10.1016/S8756-3282(99)00184-2

9. Kodama Y, Umemura Y, Nagasawa S, et al. Exercise and mechanical loading increase periosteal bone formation and whole bone strength in C57BL/6J mice but not in C3H/HeJ mice. *Calcif Tissue Int*. 2000;66(4):298–306. https://doi.org/10.1007/s002230010060
10. Judex S, Garman R, Squire M, Busa B, Donahue L-R, Rubin C. Genetically linked site-specificity of disuse osteoporosis. *J Bone Miner Res*. 2004;19(4):607–613. https://doi.org/10.1359/JBMR.040110
11. Lodberg A, Vegger JB, Jensen MV, Larsen CM, Thomsen JS, Brüel A. Immobilization induced osteopenia is strain specific in mice. *Bone Rep*. 2015;2:59–67. https://doi.org/10.1016/j.bonr.2015.04.001
12. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. *Methods*. 2001;25(4):402–408. https://doi.org/10.1006/meth.2001.1262
13. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents using micro–computed tomography. *J Bone Miner Res*. 2010;25(7):1468–1486. https://doi.org/10.1002/jbmr.141
14. Kerschan-Schindl K, Papageorgiou M, Föger-Samwald U, Butylina M, Weber M, Pietschmann P. Assessment of bone microstructure by micro CT in C57BL/6J mice for sex-specific differentiation. *IJMS*. 2022;23(23):14585. https://doi.org/10.3390/ijms232314585
15. Papageorgiou M, Föger-Samwald U, Wahl K, Kerschan-Schindl K, Pietschmann P. Age- and strain-related differences in bone microstructure and body composition during development in inbred male mouse strains. *Calcif Tissue Int*. 2020;106(4):431–443. https://doi.org/10.1007/s00223-019-00652-8
16. Föger-Samwald U, Knecht C, Stimpfl T, et al. Bone effects of binge alcohol drinking using prepubescent pigs as a model. *Alcohol Clin Exp Res*. 2018;42(11):2123–2135. https://doi.org/10.1111/acer.13874
17. Dempster DW, Compston JE, Drezner MK, et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res*. 2013;28(1):2–17. https://doi.org/10.1002/jbmr.1805
18. Idris A. *Bone Research Protocols: Third Edition. Aymen I. Idris (Editor)*, Humana Press, 2019. https://doi.org/10.1007/978-1-4939-8997-3.
19. Ganapamo F, Dennis VA, Philipp MT. Differential acquired immune responsiveness to bacterial lipoproteins in Lyme disease-resistant and -susceptible mouse strains. *Eur J Immunol*. 2003;33(7):1934–1940. https://doi.org/10.1002/eji.200323655
20. Gautam A, Dixit S, Embers M, et al. Different patterns of expression and of IL-10 modulation of inflammatory mediators from macrophages of Lyme disease-resistant and -susceptible mice. *PLoS One*. 2012;7(9):e43860. https://doi.org/10.1371/journal.pone.0043860
21. Zhang M, Yin L, Zhang K, et al. Response patterns of cytokines/chemokines in two murine strains after irradiation. *Cytokine*. 2012;58(2):169–177. https://doi.org/10.1016/j.cyto.2011.12.023
22. Matsutani T, Ananthasamy T, Kang S, Bland K, Chaudry I. Mouse genetic background influences severity of immune responses following trauma-hemorrhage. *Cytokine*. 2005;30(4):168–176. https://doi.org/10.1016/j.cyto.2004.12.019
23. Balga R, Wetterwald A, Portenier J, Dolder S, Mueller C, Hofstetter W. Tumor necrosis factor-alpha: alternative role as an inhibitor of osteoclast formation in vitro. *Bone*. 2006;39(2):325–335. https://doi.org/10.1016/j.bone.2006.02.056
24. Kim MY, Lee K, Shin H-I, Lee K-J, Jeong D. Metabolic activities affect femur and lumbar vertebrae remodeling, and antiresorptive risedronate disturbs femoral cortical bone remodeling. *Exp Mol Med*. 2021;53(1):103–114. https://doi.org/10.1038/s12276-020-00548-w
25. Akhter MP, Otero JK, Iwaniec UT, Cullen DM, Haynatzki GR, Recker RR. Differences in vertebral structure and strength of inbred female mouse strains. *J Musculoskelet Neuronal Interact*. 2004;4:33–40.
26. Föger-Samwald U, Papageorgiou M, Wahl-Figlash K, Kerschan-Schindl K, Pietschmann P. Bone and muscle development in three inbred female mouse strains. *Osteologie*. 2021;30:173–181.
27. Bouxsein ML, Myers KS, Shultz KL, Donahue LR, Rosen CJ, Beamer WG. Ovariectomy-induced bone loss varies among inbred strains of mice. *J Bone Miner Res*. 2005;20(7):1085–1092. https://doi.org/10.1359/JBMR.050307
28. Buie HR, Moore CP, Boyd SK. Postpubertal architectural developmental patterns differ between the L3 vertebra and proximal tibia in three inbred strains of mice. *J Bone Miner Res*. 2020;23(12):2048–2059. https://doi.org/10.1359/jbmr.080808
29. Akhter MP, Cullen DM, Pedersen EA, Kimmel DB, Recker RR. Bone response to in vivo mechanical loading in two breeds of mice. *Calcif Tissue Int*. 1998;63(5):442–449. https://doi.org/10.1007/s002239900554
30. Sheng MH-C, Baylink DJ, Beamer WG, Donahue LR, Lau K-HW, Wergedal JE. Regulation of bone volume is different in the metaphyses of the femur and vertebra of C3H/HeJ and C57BL/6J mice. *Bone*. 2002;30(3):486–491. https://doi.org/10.1016/S8756-3282(01)00693-7
31. Kaye M, Kusy RP. Genetic lineage, bone mass, and physical activity in mice. *Bone*. 1995;17(2):131–135. https://doi.org/10.1016/S8756-3282(00)00164-2
32. Bikle DD, Harris J, Halloran BP, Morey-Holton ER. Skeletal unloading induces resistance to insulin-like growth factor I. *J Bone Miner Res*. 1994;9(11):1789–1796. https://doi.org/10.1002/jbmr.5650091116
33. Kostenuik PJ, Harris J, Halloran BP, Turner RT, Morey-Holton ER, Bikle DD. Skeletal unloading causes resistance of osteoprogenitor cells to parathyroid hormone and to insulin-like growth factor-I. *J Bone Miner Res*. 1999;14(1):21–31. https://doi.org/10.1359/jbmr.1999.14.1.21

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