TITLE

Premature changes in trabecular and cortical microarchitecture results in decreased bone strength in hemophilia

RUNNING TITLE

Decreased bone density and strength in hemophilia

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KEY POINTS

1. Hemophilia patients have abnormal bone microarchitecture and decreased biomechanical bone strength compared to normal healthy controls.

2. Decreased bone mineral density and bone strength is associated with severity of hemophilic arthropathy.

ABSTRACT

Low bone density is a growing concern in aging hemophilia males and may result in high morbidity fragility fractures. Using high-resolution peripheral quantitative computed tomography (HR-pQCT) we demonstrate low trabecular and cortical bone density contributing to lower volumetric bone mineral density (BMD) at both distal radius and tibia in hemophilia patients compared to age- and sex-matched controls. The low trabecular bone density in hemophilia is attributed to significantly decreased trabecular number and increased separation; the lower cortical bone density results from thinner cortices, while cortical porosity is maintained. Microfinite element analysis from 3D HR-pQCT images demonstrates these microarchitectural deficits seen in the hemophilia patients translates into significantly lower estimated failure load (biomechanical bone strength) at the distal tibia and radius compared to controls. In addition, an inverse association of joint score with BMD and failure load suggests the negative role of hemophilic arthropathy in bone density loss.
INTRODUCTION

With increasing availability and affordability of safe factor concentrate replacement therapy, factor prophylaxis and comprehensive care, persons with hemophilia (PWH) today should have a normal life expectancy, and more are living beyond age 65.\textsuperscript{1,2} However, comorbid complications associated with aging in this population are largely unknown and background data on the clinical implications of these issues is lacking. Low bone mineral density (BMD) and associated osteoporosis is one of these emerging concerns that has been described with an estimated prevalence of up to 70\% in PWH.\textsuperscript{3,4,5} Although low BMD is well described in PWH, data remains limited on fracture risk with case series reporting fracture prevalence of 12-18\%.\textsuperscript{6–8}

Osteoporosis is a pathologic bone disorder characterized by low BMD and microarchitectural bone disruption that results in increased risk of fracture.\textsuperscript{9} The majority of studies investigating BMD in hemophilia males have used areal BMD measurement by dual X-ray absorptiometry (DXA) which is unable to account for geometric differences affecting true volumetric BMD.\textsuperscript{10} Furthermore, DXA provides no information on the microarchitecture of the cortical and trabecular compartments that ultimately underpins bone strength.\textsuperscript{11}

In this pilot study, we used high-resolution peripheral quantitative computed tomography (HR-pQCT) to measure true BMD, detect changes in cortical and trabecular bone microarchitecture (to an isotropic voxel size of 82\(\mu\)m), and used novel micro-finite element analysis (\(\mu\)FEA) to assess the impact of skeletal alterations on bone strength compared with matched controls. Since inactivity and cytokine-induced bone mineral loss due to chronic arthropathy are proposed
mechanisms for BMD loss in PWH,12,13 Gilbert joint scores for severity of hemophilia arthropathy were measured for correlation analysis.14

STUDY DESIGN

Subjects

After obtaining University of Calgary Research Ethics Board approval, 18 Hemophilia A and B subjects (≥ 18 years-old) with severe (factors VIII or IX <1 U/dL) or moderate (FVIII/IX 1-5 U/dL) disease were recruited through the Southern Alberta Rare Blood and Bleeding Disorders (RBDD) Comprehensive Care Program. Subjects receiving corticosteroids for greater than 3 months were excluded. HR-pQCT data on age- and sex- matched controls (at 2:1 ratio to hemophilia patients) was obtained from the Canadian Multicentre Osteoporosis Study (CaMos) on healthy subjects.15

Protocol

All procedures were conducted at the Southern Alberta RBBD Program and the University of Calgary Bone Imaging Laboratory. On each subject, anthropomorphic measurements and Gilbert joint score were collected. Gilbert joint score, a measure for severity of joint arthropathy, reflects the combined score of six joints (elbows, knees, ankles) where higher score indicates worse arthropathy and was performed by a single, expert-trained examiner (AL) for all subjects.16 Bone microarchitecture and strength of the non-dominant distal radius and tibia were assessed by HR-pQCT (XtremeCT, Scanco Medical) and µFEA (Faim v6.0, Numerics88 Solution Ltd, Calgary).
HR-pQCT imaging

Distal radius and tibia HR-pQCT measurements and analysis of hemophilia subjects were performed identically to the CaMos study. Standard morphologic analysis include: total volumetric BMD (mg HA/cm³), ratio of trabecular bone volume to total bone volume (BV/TV, reflecting trabecular BMD), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), and trabecular separation (Tb.Sp, mm); as well as cross-sectional cortical analysis – cortical BMD (Ct.BMD, mg HA/cm³), cortical thickness (Ct.Th, mm), and cortical porosity (Ct.Po, %).

μFEA

Linear homogenous finite element models from 3D HR-pQCT image data were generated as per the CaMOS study. Finite element models were solved using μFEA software to calculate estimated biomechanical bone strength or failure load (Newtons, N), which is the amount of axial force needed to be applied to the bone for it to fail.

Statistical Analysis

Analysis of variance model adjusted for age was used for comparison of bone parameters between hemophilia patients and controls. Significance is defined as p<0.05. Spearman rank correlation analysis was used to investigate the relationship between BMD, failure load, and Gilbert joint score.
RESULTS AND DISCUSSION

As indicated in Table 1, eighteen moderate or severe hemophilia patients were included in this pilot study; 15 (83%) with Hemophilia A. Amongst them, 15 (83%) have severe disease, 3 (17%) are HIV positive and 4 (22%) have documented liver cirrhosis. Only five (28%) had received primary prophylaxis. The control and hemophilia groups had similar average age (33.5 vs. 33.7 years), whereas the controls had insignificantly higher height and weight, and lower BMI.

Vitamin D levels were available on 17 patients; 41% had vitamin D deficiency (<50nmol/L).

Compared to matched controls, PWH demonstrated significantly lower non-dominant distal tibia and radius total BMD (288.9 and 313 mg HA/cm³; \( p=0.003, p=0.012 \)), trabecular BMD (BV/TV: 0.15 and 0.14; \( p=0.004, p=0.014 \)) and cortical BMD (880 and 958 mg HA/cm³; \( p=0.032, p=0.003 \)). In addition, hemophilia patients had significantly lower trabecular number (Tb.N) in the distal tibia but not radius, and increased trabecular separation (Tb.Sp) at both tibia and radius. Cortical thickness (Ct.Th) was significantly lower at both sites, but not cortical porosity (Ct.Po).

The 3D reconstructed image of the distal radius of a severe Hemophilia A subject illustrates fewer trabeculae which are less dense as well as a thinner cortex compared to the matched healthy control. (Figure 1A)

μFEA assessment of the reconstructed HR-pQCT images indicates that the lower BMD and microarchitectural deficits in the hemophilia patients translated into significantly lower failure load at the distal tibia (6357 versus 7573 N; \( p=0.009 \)) and radius (2371 vs. 3013 N; \( p=0.002 \)) compared to matched controls (Figure 1B).
There is a significant inverse correlation of Gilbert joint score to tibia BMD (R= -0.697, p=0.002), tibia failure load (R= -0.635, p=0.006), and radius failure load (R= -0.552, p=0.022) (Figure 1C). An inverse relationship with Gilbert score is seen for radius BMD (R= -0.372, p=0.142) though not statistically significant. Individuals who received primary prophylaxis appear to have higher failure load.

These compartmental alterations in microarchitecture in PWH are similar to those reported in post-menopausal women with osteoporosis and fragility fractures; specifically lower trabecular BMD and fewer Tb.N. Decreased CtPo, described in post-menopausal women, was not seen in the hemophilia subjects.\textsuperscript{18,19} Cortical thinning and trabecular bone loss are known to be important contributors to bone fragility; while low cortical porosity appears to be the main contributor to low bone strength in patients with monoclonal gammopathy of unknown significance.\textsuperscript{20} Studies in fractured osteopenic women demonstrate that lower trabecular BMD (BV/TV) due to loss of Tb.N (as opposed to decreased Tb.Th) and increased Tb.Sp are important contributors to low bone strength (2-5 times by finite element method).\textsuperscript{21,22} Population studies using HR-pQCT and \(\mu\)FEA demonstrate progressive cortical thinning and porosity, and loss of trabecular number leads to decreased bone strength with aging.\textsuperscript{23,24} These findings in our hemophilia study subjects is concerning considering their average age was only 33.5 years old. Low trabecular BMD specifically at the distal radius has also been described in hemophilia boys (6.6-19.8 years) using lower resolution pQCT\textsuperscript{25}. Taken together, these findings suggest microarchitectural deficits start at an early age in hemophilia and poor bone strength can be expected to worsen over time.
The mechanism for low BMD and osteoporosis in hemophilia is multifactorial. One of the hypothesized contributors to this process is severity of hemophilic arthropathy which may result in decreased axial loading due to inactivity, and/or inflammatory cytokine-induced bone resorption. This study suggests that hemophilic arthropathy results in greater BMD loss, as shown by the inverse association of joint score with BMD and failure load at both the tibia and radius.

Limitations of this study are the sample size and inability to perform subgroup analysis. Also, the HR-pQCT data from this study is unable to describe the dynamic processes underlying the bone pathophysiology and whether there is excessive osteoclast mediated resorption, decreased osteoblastic new bone formation or both. Future inclusion of bone remodeling indices may help clarify the mechanisms and be pertinent to proposed interventions. Our data, however, is the first to our knowledge to describe abnormal microarchitectural changes in both cortical and trabecular compartments, and significantly reduced bone strength associated with severity of hemophilic arthropathy in adult hemophilia patients. Further studies are needed to determine if estimated bone strength using HR-pQCT is predictive of future fracture risk as these patients age.

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Raymond, and Anne Cooke from the University of Calgary Bone Imaging Lab for performing HR-pQCT scanning and μFEA calculations.

Contributions of authors

Drs Adrienne Lee, Stephen Boyd, Gregory Kline, and Man-Chiu Poon designed the study. Dr. Stephen Boyd supervised the HR-pQCT scans and μFEA calculations performed at the University of Calgary Bone Imaging Laboratory, and provided control data from the CaMOS study. Dr Adrienne Lee performed the statistical analysis and wrote the manuscript for this paper, and all authors participated in its editing and revision. The authors have no competing interests.

REFERENCES


Table 1. Clinical characteristics and distal tibia and radius bone parameters and biomechanical bone strength (derived from μFEA of HR-pQCT images)

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Hemophilia (n=18)</th>
<th>Control (n=36)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Male, n (%)</td>
<td>18 (100)</td>
<td>36 (100)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>33.5 ± 3.1</td>
<td>33.7 ± 2.2</td>
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<tr>
<td>Height (cm)</td>
<td>167.2 ± 6.8</td>
<td>177.6 ± 1.2</td>
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<tr>
<td>Weight (kg)</td>
<td>70.7 ± 5.6</td>
<td>78.1 ± 2.2</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>26.5 ± 2.4</td>
<td>24.8 ± 0.8</td>
<td>0.512</td>
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<td>HIV positive, n (%)</td>
<td>3 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilia A/B, n (%)</td>
<td>15 (83)/3 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe/moderate hemophilia, n (%)</td>
<td>15 (83)/3 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis, n (%)</td>
<td>4 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary prophylaxis, n (%)</td>
<td>5 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive fracture history, n (%)</td>
<td>4 (22)</td>
<td></td>
<td></td>
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<tr>
<td>*Vitamin D (nmol/L)</td>
<td>59.1 ± 6.5</td>
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<tr>
<td>¥Gilbert Joint score</td>
<td>15.7 ± 2.8</td>
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<tr>
<th>HR-pQCT Bone Parameters</th>
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<th>Radius</th>
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<tr>
<td>BMD (mg HA/cm³)</td>
<td>Hemophilia</td>
<td>Control</td>
<td>P value</td>
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<tr>
<td>Trabecular Parameters</td>
<td>288.9 ± 15.8</td>
<td>346.7 ± 7.3</td>
<td>0.003</td>
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<td>BV/TV</td>
<td>0.15 ± 0.008</td>
<td>0.18 ± 0.005</td>
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<td>Tb.N (1/mm)</td>
<td>1.7 ± 0.05</td>
<td>2.0 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>0.1 ± 0.005</td>
<td>0.09 ± 0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Tb.Sp (mm)</td>
<td>0.52 ± 0.02</td>
<td>0.42 ± 0.01</td>
<td>&lt;0.001</td>
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<tr>
<td>Cortical parameters</td>
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<tr>
<td>Ct.BMD (mg HA/cm³)</td>
<td>880 ± 19.9</td>
<td>928 ± 5.4</td>
<td>0.032</td>
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<tr>
<td>Ct.Th (mm)</td>
<td>1.3 ± 0.07</td>
<td>1.5 ± 0.04</td>
<td>0.049</td>
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<tr>
<td>Ct.Po (%)</td>
<td>5.9 ± 0.87</td>
<td>4.9 ± 0.27</td>
<td>NS</td>
</tr>
</tbody>
</table>

| Biomechanical Bone Strength (derived from μFEA)         |                   |                |         |
| Estimated Failure Load (N)                             | 6357 ± 377        | 7573 ± 217     | 0.009   | 2371 ± 162 | 3013 ± 90 | 0.002   |

Unless otherwise specified, values are presented as percent or mean ± SE. Comparisons of bone parameters are adjusted for age and gender.

BMI=body mass index; BMD=total volumetric bone mineral density; BV/TV=trabecular bone volume to total volume ratio (a measure of trabecular bone mineral density); Tb.N=trabecular number; Tb.Th=trabecular thickness; Tb.Sp=trabecular separation; Ct.BMD=cortical bone mineral density; Ct.Th=cortical thickness; Ct.Po=cortical porosity; μFEA=micro-finite element analysis; HA=hydroxyapatite; N=newton.

*Vitamin D levels available in 17 subjects; 7 (41%) vitamin D deficiency (<50nmol/L).

¥ Gilbert joint score measured in 17 subjects.
Figure 1. Bone microarchitecture, bone strength and correlation with joint score. (A) Representative HR-pQCT images at a nominal isotropic resolution of 82 μm of the distal radius in a 43 year old with severe hemophilia A male patient (TbN 1.82, TbTh 0.061, CtTh 0.94, CtPo 2.23) and an age- and sex-matched control subject (TbN 2.02, TbTh 0.07, CtTh 1.12, CtPo 1.18). (B) Failure load (higher failure load = better mechanical bone strength) at the distal tibia in hemophilia patients and controls. The upper and lower boundary of the box plot representing the IQR, diamond representing the mean, the line dividing the box plot representing the median, and whiskers indicate the maximum and minimum values. (C) Failure load at distal tibia versus total Gilbert joint score (higher joint score = more severe arthropathy) in 17 hemophilia patients (the remaining one patient did not have Gilbert joint score available). Open square data points (□) indicate subjects who received primary prophylaxis (only 4 of 5 on primary prophylaxis shown as one did not have joint score available) and as a whole appears to have lower Gilbert joint scores and higher failure load. The outlier (X) with the lowest tibial failure load has liver cirrhosis secondary to HCV infection and HIV which likely explains the lower tibial failure load and BMD. When the outlier is excluded from the correlation analysis, the statistically significant inverse correlation of Gilbert joint score and tibial BMD and failure load still stands (tibial failure load: $R=-0.601$, $p=0.0139$; tibial BMD: $R=-0.66$, $p=0.005$). $R =$ Spearman $\rho$; $p = P$ value.
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