

# Long-term bisphosphonate treatment coupled with ovariectomy in mice provokes deleterious effects on femoral neck fracture pattern and modifies tibial shape



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## Aims

The processes linking long-term bisphosphonate treatment to atypical fracture remain elusive. To establish a means of exploring this link, we have examined how long-term bisphosphonate treatment with prior ovariectomy modifies femur fracture behaviour and tibia mass and shape in murine bones.

## Methods

Three groups (seven per group) of 12-week-old mice were: 1) ovariectomized and 20 weeks thereafter treated weekly for 24 weeks with 100 µm/kg subcutaneous ibandronate (OVX+IBN); 2) ovariectomized (OVX); or 3) sham-operated (SHAM). Quantitative fracture analysis generated biomechanical properties for the femoral neck. Tibiae were microCT scanned and trabecular (proximal metaphysis) and cortical parameters along almost its whole length measured.

## Results

Fracture analyses revealed that OVX+IBN significantly reduced yield displacement (vs SHAM/OVX) and resilience, and increased stiffness (vs SHAM). OVX+IBN elevated tibial trabecular parameters and also increased cortical cross-sectional area and second moment of area around minor axis, and diminished ellipticity proximally.

## Conclusion

These data indicate that combined ovariectomy and bisphosphonate generates cortical changes linked with greater bone brittleness and modified fracture characteristics, which may provide a basis in mice for interrogating the mechanisms and genetics of atypical fracture aetiology.

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## Introduction

Atypical fractures (AF) are a rare yet catastrophic phenomenon involving failure following minimal bone trauma. Although incidence correlates strongly with long-term bisphosphonate use, AF pathophysiology is poorly described, and treatment and prevention strategies are elusive.<sup>1-4</sup> It is speculated that suppression of remodelling by long-term bisphosphonate use increases bone brittleness, resulting in inefficient dissipation of the energy imposed by even relatively

minor loads and hence crack propagation and fracture.<sup>1-3,5</sup>

Bisphosphonates inhibit resorption and can rapidly modify bone architecture and quality.<sup>6,7</sup> Use in rodents has demonstrated their role in preserving cortical and trabecular microstructure.<sup>8,9</sup> Their prolonged administration can, however, generate contrary outcomes, with brittle bone susceptible to failure with minimal deformation.<sup>1,2,5,7,10</sup> There are various explanations for bisphosphonate-related weakening;

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observations in osteoporotic patients suggest that long-term bisphosphonate administration impairs osteoclast-mediated repair of microdamage to promote microcrack accumulation.<sup>2,3,5,11</sup> Treatment is also likely to influence the bone's properties directly, increasing mineralization levels and altering intrinsic resistance to bending without failure.<sup>1,2,4</sup> Bisphosphonates induce similar deleterious energy absorption and toughness reductions in rat femoral bone and alter bone mechanical properties in dog models.<sup>4,10</sup> Suppression of bone turnover in these models also leads to bone matrix collagen crosslinking to negatively impact biomechanical properties, particularly post-yield deformation.<sup>4,12</sup>

Clinical observations in bisphosphonate-treated patients highlight that changes in bone shape could be another risk factor,<sup>1,2</sup> as have specific gene polymorphisms in human cohort-based analyses.<sup>13</sup> There are reports of modified geometry and increased femoral bowing, which influence local mechanical strain directly,<sup>1,2,14</sup> emphasizing the role of modelling activity in maintaining shape and mechanical integrity of normal, fracture-resistant bone.<sup>15</sup> The precise shape changes driven by long-term bisphosphonates, and their mechanical consequences, need therefore to be explored to gain a thorough understanding of AF susceptibility.<sup>2,14,15</sup> Imaging advances now allow investigation of subtle changes in murine tibia shape,<sup>16,17</sup> and their relevance to age-related bone loss in humans has been emphasized.<sup>16</sup> This makes the study of bone shape appealing, particularly in the experimental context where bisphosphonates may also provoke AF. Clinical observations would clearly be enriched by *in vivo* experiments in mice where all the stages predisposing to AF development could be studied.

AF is currently only limited by imposing a bisphosphonate 'drug holiday' for microcrack repair and shape correction. An animal model that compellingly recapitulates events which contribute to AF would clearly improve prevention and treatment strategies, and would provide a basis from which gene polymorphisms.<sup>18,19</sup> Herein, we address the hypothesis that long-term bisphosphonate exposure in ovariectomized mice provokes changes in bone strength, quantity and geometry that effectively mimic those culminating in AF susceptibility. We examine whether such treatment provokes bone strength changes that culminate in modified fracture behaviours in an established model of ovariectomy-induced osteoporosis, and whether this combined treatment induces tibia shape changes consistent with an increased mechanical fracture vulnerability.

## Methods

**Animals, anaesthesia, surgery, and treatment.** In all, 21 12-week-old female Swiss mice were randomly allocated into three groups (seven per group), housed in groups

of seven in polypropylene cages with temperature maintained between 19°C to 23°C, 12-hour light/dark cycle with ad libitum maintenance mice diet (rats and mice, Presence, Paulinia, Brazil) and water. Surgery involved pre-medication with 10 mg/kg meperidine (Dolosal 50 mg/ml; Cristália Itapira, Brazil), 2 mg/kg meloxicam (Maxican 2%; Ourofino Pet Cravinhos, Brazil) delivered subcutaneously with isoflurane-induced anaesthesia (Isoforine; Cristália Itapira, Brazil), followed by right flank incision and abdominal cavity access without ovariectomy (SHAM), removal of ovaries (OVX), or ovary removal followed 20 weeks post-surgery (aged 32 weeks) by an additional 24-week ibandronate treatment (OVX+IBN). SHAM and OVX groups received weekly subcutaneous saline injection, while OVX+IBN received 100 µg/kg weekly subcutaneous ibandronate (Bonviva 3 mg/3 ml; Roche, Rio de Janeiro, Brazil).<sup>18,20</sup> All mice were killed by cervical dislocation aged 56 weeks, weighed and the femur and right tibia subsequently dissected free of soft tissue and frozen (-80°C) or fixed in neutral-buffered formaldehyde for 24 hours before storage in 70% EtOH, respectively.

**Assessment of femoral head biomechanical properties.** Femoral neck strength was evaluated by compression until failure, using established methods.<sup>21,22</sup> Briefly, after defrosting, the distal half of the femur was dissected and the proximal segment diaphysis embedded in acrylic base, allowing loading of femur head parallel to the diaphysis (Figure 1).<sup>22</sup> Fracture tests were performed in a universal testing machine (Instron Model 5565, Canton, Massachusetts, USA) using a cylindrical jig with the femoral head travelling at 0.5 mm/min until neck failure with 2 Hz data sampling. Displacement and force were recorded and yield displacement (YD), stiffness, ultimate load (UL) before fracture and resilience calculated for each sample. Stiffness was evaluated from the linear elastic phase of the force-displacement curves (angular coefficient,  $R^2 > 0.99$  was considered appropriate) and resilience, via YD and force (resilience =  $(YD \times \text{yield force})/2$ ).

**Assessment of bone mass and shape changes in tibial cortical and trabecular compartments.** To evaluate treatment effects on bone shape and mass, each entire tibia was scanned using radiograph microcomputed tomography (microCT; Skyscan 1172, Kontich, Belgium) with 0.5 mm aluminium filter, medium camera, 5 µm voxel size, tube operated at 50kv/200µa and 960 ms exposure time. Slices were reconstructed using NRecon 1.7.1.0 and length measured with CTAn software. Cortical bone quantity and shape along the tibia length (15% to 85%, proximodistally) was also evaluated.<sup>17</sup> After alignment in DataViewer, images were imported to ImageJ and the fibula removed. BoneJ plugin was used (grayscale threshold of 100) to measure cross-sectional area (CSA), average cortical thickness (Ct.Th), second moment of area around major/minor axes ( $I_{\min}/I_{\max}$ ), predicted resistance

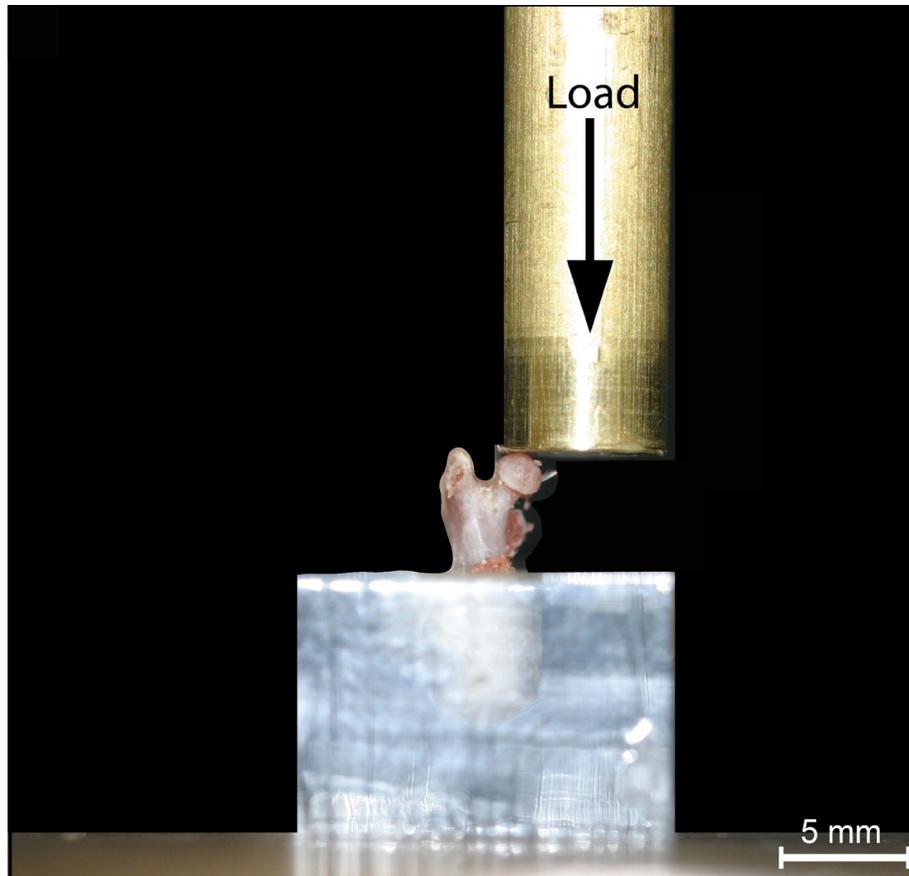


Fig. 1

The positioning of the femur in the universal materials testing machine prior to compression until failure. The black arrow represents the direction of force applied.

to torsion (J) and ellipticity. Tissue mineral density (TMD) was measured following appropriate calibration.

For trabecular bone, the appearance of the trabecular 'bridge' connecting the paired primary spongiosa 'islands' was set as the reference point for analysis of proximal metaphyseal bone; 5% of total bone length (from this point towards diaphysis) was selected for evaluation of trabecular number (Tb.N), thickness (Tb.Th), separation (Tb.Sp), bone volume/trabecular volume (BV/TV) and mineral density (BMD), by calibrated microCT.<sup>17</sup>

**Statistical analysis.** All data (except body mass and length) were weight-corrected using a linear regression method,<sup>23,24</sup> tested by Shapiro-Wilk to verify adherence to normal distribution and differences in body mass, femoral YD, stiffness, UL, and resilience, and tibia trabecular parameters evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Parameters failing normality tests ( $p < 0.05$ ) were analyzed by Kruskal-Wallis test followed by a Dunn's test. All analyses were performed using Prism 7 (GraphPad Software, San Diego, California, USA) and significance set at  $p < 0.05$ . Tibia cortical parameters were assessed by one-way ANOVA followed by Tukey's post-test (R software, version

3.4.4.4) and distinct levels of significance set at  $p < 0.001$ ,  $0.001 < p < 0.01$  and  $0.01 < p < 0.05$ .

## Results

Tibia length measurements showed that treatment did not modify the extent of longitudinal growth during the 44-week study duration (SHAM, OVX, and OVX+IBN were  $20.08 \text{ mm} \pm 0.12 \text{ mm}$ ,  $20.23 \text{ mm} \pm 0.16 \text{ mm}$ , and  $20.27 \text{ mm} \pm 0.14 \text{ mm}$  respectively). OVX and particularly OVX+IBN groups did, however, show a tendency for raised body mass (SHAM, OVX, and OVX+IBN were  $43.86 \text{ gm} \pm 2.83 \text{ gm}$ ,  $50.57 \text{ gm} \pm 3.1 \text{ gm}$ , and  $52.71 \text{ gm} \pm 0.96 \text{ gm}$  respectively); accordingly, all further evaluations were subjected to weight-correction using a linear regression method.<sup>23</sup>

**Combined ibandronate/ovariectomy increases femoral brittleness.** Evaluation of force/displacement curves from a compressive load fracture method showed that femora in the OVX+IBN group exhibited ~40% lower YD (vs SHAM/OVX, reduction) and greater stiffness (vs SHAM; OVX vs SHAM not different; Figure 2a and b). Ultimate (maximum) load-to-fracture – a measure of strength – did not differ in OVX or OVX+IBN groups

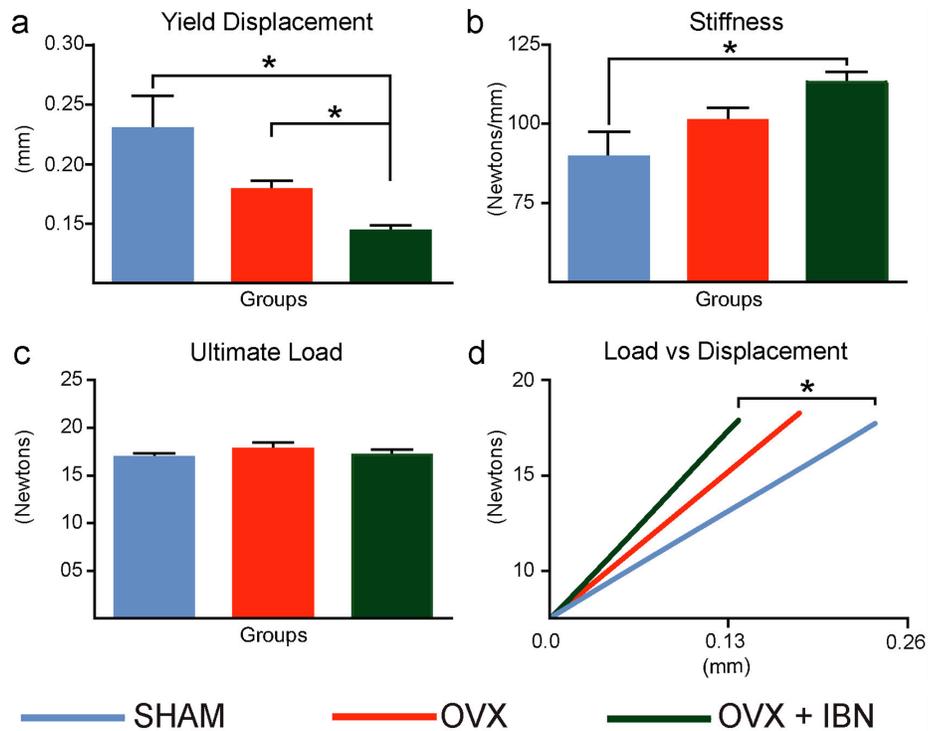


Fig. 2

Effect of ibandronate/ovariectomy treatment on biomechanical properties. Measurement (mean  $\pm$  SE) of yield displacement (mm): (a) stiffness (Newtons/mm), (b), ultimate load (Newtons, (c) and average load, displayed as a function of displacement, across a linear portion of the load/displacement curve (d) in mouse femoral neck fracture model in sham-operated mice (SHAM), ovariectomized (OVX) mice receiving subcutaneous saline solution and ovariectomized mice treated weekly, from 20 weeks, for 24 weeks with subcutaneous ibandronate (Bonviva, 1 mg/ml, Roche, 100  $\mu$ m/kg; OVX+IBN). Group size was seven per group. Stiffness corresponded to the Shapiro-Wilk test for normality and was analyzed through ANOVA followed by Tukey test. Yield displacement and ultimate load failed to normality tests ( $p < 0.05$ ) and were analyzed by Kruskal-Wallis test followed by a Dunn's test. \*Statistical significance ( $p < 0.05$ ).

compared to SHAM (Figure 2c). Consistent with greater brittleness, OVX+IBN treatment significantly elevates neck stiffness (vs SHAM; Figure 2d). Femur resilience was also significantly lower (~47% reduction) in OVX+IBN than SHAM group (Figure 3).

**Combined ibandronate/ovariectomy modifies cortical mass and shape in specific tibia regions.** Evaluation along tibial length revealed that OVX+IBN elevated cortical CSA (vs OVX) in the proximal diaphysis (~20% to 30%; Figure 4a). In contrast, neither Ct.Th nor TMD were modified by OVX+IBN or OVX treatment. Superimposition of changes at 35% of length highlight cortical shape changes between SHAM, OVX and OVX+IBN groups (Figure 4b); while  $I_{\min}$  and  $J$  did not differ across these groups,  $I_{\max}$  was significantly greater, and ellipticity lower along proximal regions in OVX+IBN mice (~30% to 45% vs OVX/SHAM; Figure 5a and b). These data indicate that OVX+IBN generates greater cortical mass and straighter shape at localized tibial regions.

**Combined ibandronate/ovariectomy increases trabecular mass and mineral density.** Trabecular architecture was not significantly modified 44 weeks after ovariectomy (vs SHAM) and only Tb.Sp was significantly different in OVX+IBN compared with SHAM (Table I). In contrast,

OVX+IBN resulted in significantly greater BV/TV, Tb.N and BMD, and less Tb.Sp than was evident on OVX mice.

## Discussion

Our data show that combined OVX+IBN treatment reduces YD and resilience, and increases femoral stiffness/brittleness without modifying ultimate load. We also report greater tibial CSA and  $I_{\max}$ , and decreased ellipticity proximal to the mid-shaft in OVX+IBN treated mice, consistent with regional cortical mass and shape modification. OVX-induced trabecular bone loss was, as expected, restricted by additional IBN treatment. Emergence of greater brittleness, cortical thickening, and shape changes make it tempting to speculate that OVX+IBN treatment in mice allows for the study of AF-type pathophysiology.

These skeletal deficiencies emerge 20 weeks after OVX, followed by an additional 24-week ibandronate treatment period. Despite immaturity at study onset, mice will have attained skeletal maturity while estrogen-deplete, before ibandronate treatment, which only finishes at ~one-year-old.<sup>16</sup> It is difficult to fully reconcile our observations as studies into the influence of bisphosphonates on mouse bone fracture properties are limited. Elegant studies by Aref et al<sup>25</sup> used femoral four-point bending

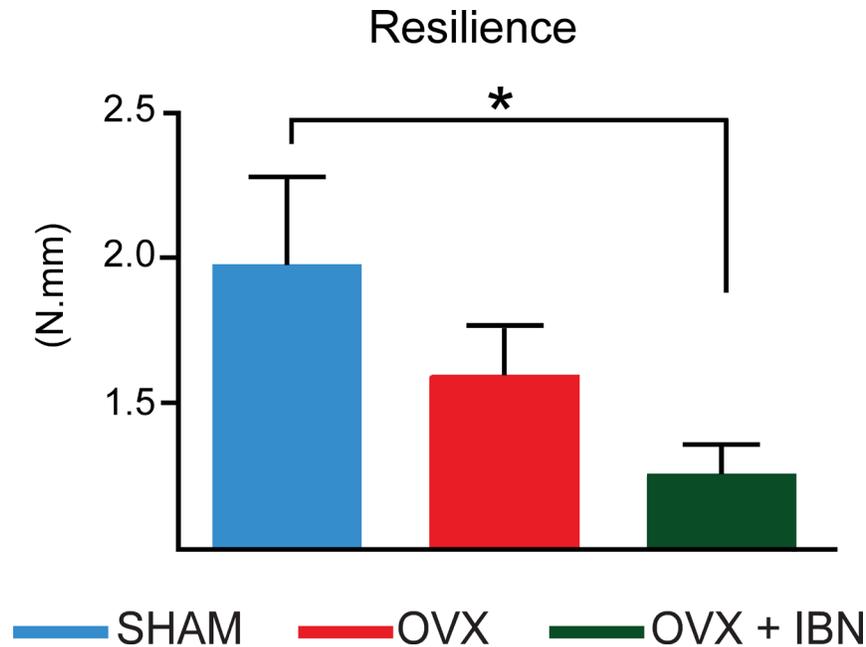


Fig. 3

Effect of ibandronate/ovariectomy treatment on biomechanical properties. Measurement (mean  $\pm$  SE) of resilience (N.mm) in mouse femoral neck fracture model in sham-operated mice (SHAM), ovariectomized (OVX) mice receiving subcutaneous saline solution and ovariectomized mice treated weekly, from 20 weeks, for 24 weeks with subcutaneous ibandronate (Bonviva®, 1 mg/ml, Roche, 100  $\mu$ m/kg; OVX+IBN). Group size was seven per group. Resilience failed to normality tests ( $p < 0.05$ ) and was analyzed by Kruskal-Wallis test followed by a Dunn's test. \*Statistical significance ( $p < 0.05$ ).

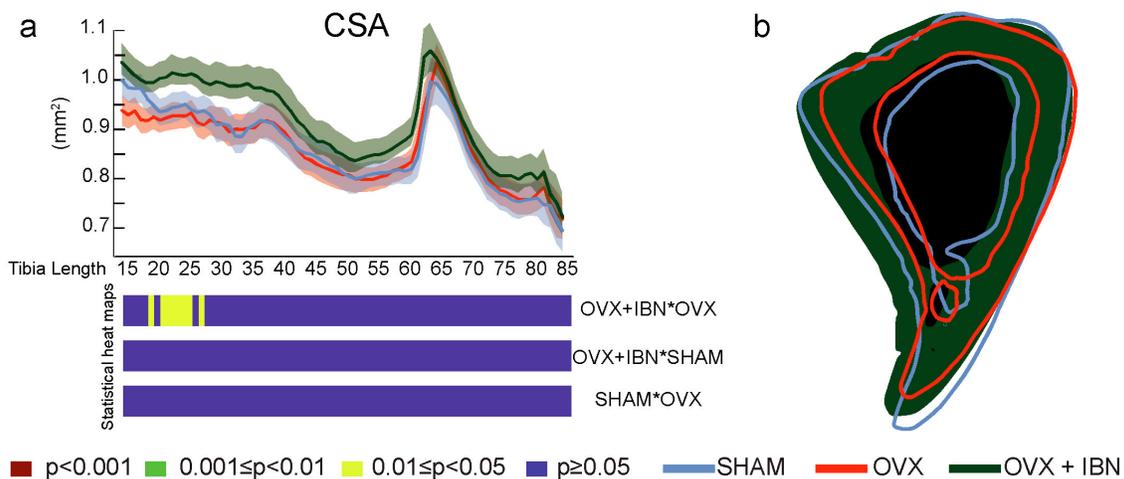


Fig. 4

Effects of ibandronate/ovariectomy treatment on cortical mass in the tibia. Measurement (mean  $\pm$  SE) and *t*-test heat map of cross-sectional area (CSA, mm<sup>2</sup>, a) in mouse tibia along its diaphyseal length (15% to 85%). b: slice at 35% of length of representative tibia from sham-operated mice (SHAM, blue), ovariectomized (OVX, red) mice receiving subcutaneous saline solution and ovariectomized mice treated weekly, from 20 weeks, for 24 weeks with subcutaneous ibandronate (Bonviva, 1 mg/ml, Roche, 100  $\mu$ m/kg; OVX + IBN, green). Group size was seven per group; All data corresponded to the Shapiro-Wilk test for normality and was analyzed through ANOVA followed by Tukey test. Levels of statistical significance set at  $p < 0.001$  (red),  $0.001 < p < 0.01$  (green) and  $0.01 < p < 0.05$  (yellow);  $p > 0.05$  (blue).

to explore if bone properties were modified by zoledronate in divergent mouse strains, finding that C57/Bl6 but not A/J mice exhibit changes in YD.<sup>25</sup> As C57/Bl6 also exhibited greater femoral bone area, it was suggested that geometry was a determinant of bone's response to

bisphosphonate. Our studies support this conclusion, demonstrating that OVX+IBN increases brittleness and reduces femoral YD more than OVX alone.

It is noteworthy that our studies apply loads parallel to the diaphysis and thus evaluate femoral biomechanical

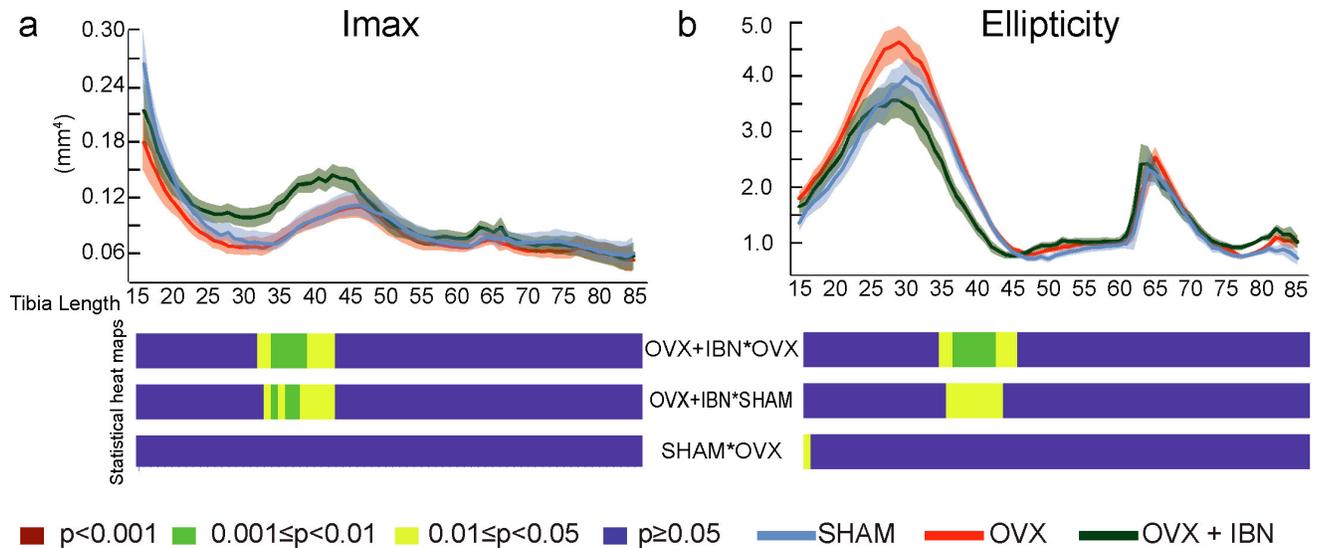


Fig. 5

Effect of ibandronate/ovariectomy treatment on the cortical bone shape in the tibia. Measurement (mean  $\pm$  SE) and *t*-test heat map for second moment of area around minor axes ( $I_{\max}$ , mm<sup>4</sup>, a) and ellipticity (b) at 15% to 85% of tibia length in sham-operated mice (SHAM), ovariectomized mice receiving subcutaneous saline solution (OVX) and ovariectomized mice treated weekly, from 20 weeks, for 24 weeks with subcutaneous ibandronate (Bonviva®, 1 mg/ml, Roche, 100  $\mu$ m/kg; OVX+IBN). Group size was seven per group; All data corresponded to the Shapiro-Wilk test for normality and was analyzed through ANOVA followed by Tukey test. Levels of statistical significance set at  $p < 0.001$  (red),  $0.001 < p < 0.01$  (green) and  $0.01 < p < 0.05$  (yellow);  $p > 0.05$  (blue).

**Table 1.** Combined ibandronate/ovariectomy treatment increases trabecular bone mass and mineral density.

Parameter	SHAM, mean $\pm$ SE	OVX, mean $\pm$ SE	OVX+IBN, mean $\pm$ SE
Tb.N (mm <sup>-1</sup> )	1.04 $\pm$ 0.23*†	0.89 $\pm$ 0.30*	1.33 $\pm$ 0.24†
Tb.Th (mm)	0.05 $\pm$ 0.00*	0.05 $\pm$ 0.00*	0.05 $\pm$ 0.00*
Tb.Sp (mm)	0.48 $\pm$ 0.07*	0.51 $\pm$ 0.07*	0.33 $\pm$ 0.05†
BV/TV (%)	5.81 $\pm$ 1.46*†	5.05 $\pm$ 1.47*	7.39 $\pm$ 1.26†
BMD (g/cm <sup>3</sup> )	0.09 $\pm$ 0.03*†	0.07 $\pm$ 0.02*	0.12 $\pm$ 0.01†

Group size seven per group. Mice receiving subcutaneous saline solution and ovariectomized mice treated weekly, from 20 weeks, for 24 weeks with subcutaneous ibandronate (Bonviva, 1 mg/ml, Roche, 100  $\mu$ m/kg; OVX+IBN). All data corresponded to the Shapiro-Wilk test for normality and was analyzed through ANOVA followed by Tukey test. Groups annotated with the same symbol are not statistically different;  $p < 0.05$  in groups annotated with different symbols.

BMD, bone mineral density; BV/TV, bone volume/trabecular volume; OVX, ovariectomized; SE, standard error; SHAM, sham-operated mice; Tb.N, trabecular number; Tb.Sp, trabecular separations; Tb.Th, trabecular thickness.

properties under more physiological conditions.<sup>21,22</sup> We tentatively suggest that OVX+IBN will help identify strength/geometry changes that occur during AF development. If mouse genotype is a major determinant,<sup>25,26</sup> future studies would ideally examine bisphosphonate responses in divergent strains and endorse a genetic predisposition to AF.<sup>13</sup>

Lower YD and resilience with greater stiffness after OVX+IBN is consistent with the greater brittleness reported after long-term bisphosphonate treatment in humans.<sup>1,5,11</sup> They are also in keeping with AF following long-term ibandronate in other patient groups;<sup>27,28</sup> with

femoral neck AF after long-term risedronate delivery<sup>29</sup> and the notion that highly mineralised bone has less toughness and faster fracture propagation.<sup>1,5,11,30</sup> This may be an amalgamation of mineral content, mass, and shape changes, and since we were not able to microCT the femora, we cannot rule out shape change contributions to OVX+IBN-related alterations in fracture characteristics. Examination of rat femur fracture properties after ovariectomy demonstrates that stiffness changes could be metabolically underpinned.<sup>21</sup> It is therefore conceivable that associated bone shape changes will be discernible elsewhere in the skeleton. Our studies show that there are indeed tibial shape changes, with greater CSA and  $I_{\max}$  following OVX+IBN. Future studies will need to correlate changes in femoral brittleness to analysis of femoral neck bone mass and shape after OVX+IBN treatment, and also include studies in SHAM-IBN mice in order to allow for more definitive conclusions to be drawn.

Likely impact of tibia-femoral angles in femur AF led us to explore tibia architectural changes with OVX+IBN.<sup>14</sup> Bone shape deviation can result from modified mechanical strains, pharmacological influences on modelling or pathology.<sup>15,31</sup> Indeed, bisphosphonates can alter mouse bone modelling, leading to femoral cortex shape changes.<sup>18</sup> In agreement, we find that OVX+IBN generates greater CSA and  $I_{\max}$ , as well as lower ellipticity (straightening) – shape changes linked to lower load predictability.<sup>32</sup> Finite element analyses have shown that the site of AF in human femora coincides with region of high strain.<sup>33</sup> It is intriguing that the tibia shape changes we

find also coincide with reported high strain regions.<sup>34</sup> As human femora with particular shape profiles are predisposed to AF,<sup>1,2,33</sup> we speculate that the shape changes after OVX+IBN likewise reduce load predictability to increase fracture risk, affording a model for checking new therapies to limit AF development.<sup>19</sup> OVX+IBN treatment of murine models with known genetic AF predisposition coupled with the monitoring of these shape changes may help to refine our understanding of AF risks.

We also examined proximal tibia metaphysis trabecular bone; a focus region for many studies. Song et al<sup>35</sup> found most marked effects after eight to 16 weeks of OVX in this region (in eight-week-old mice), which were much less marked later, suggesting that prolongation does not expand the bone losses. Advanced age and the 44-week timescale post-OVX<sup>16,35</sup> in our study may not, therefore, be ideal and may underpin the lack of any overt OVX-induced difference. It is key, however, that we evaluated long-term bisphosphonate effects as our aim was to discern whether this aligned with clinical observations of modified strength and fracture susceptibility. In osteoporotic patients, bisphosphonates limit remodelling to restrict further losses in bone mass.<sup>6,7</sup> This aligns with ibandronate-related protection against OVX-induced bone changes in both femora and vertebrae in aged rats.<sup>9</sup> In our study, ibandronate delivery commencing 20 weeks after OVX similarly sustains trabecular number, BV/TV and BMD at SHAM levels; consistent with the notion that ibandronate limits rapid declines in mass and quality that follow OVX.

The methods used to measure bone mass and mechanical properties are of central importance; for example, both are influenced by body mass.<sup>23,24</sup> We have used the methods of Jepsen et al<sup>23</sup> to control for body mass, his normalization employs linear regression to correct for even small mass differences,<sup>23</sup> and is precautionary since tibia length is not modified between groups and correction based upon length would be superfluous. We studied responses to compressive load in the femoral neck and performed whole bone microCT analysis in tibiae, as the latter aligned with previous studies examining response to pharmacological treatment.<sup>22</sup>

There are several caveats pertinent to our findings. First, it is difficult to fully appreciate the OVX+IBN effects, without a group treated with IBN alone and the lack of trabecular or cortical changes in OVX mice. Our aim, however, was to recapitulate post-menopausal osteopenia treated clinically with long-term ibandronate, rather than to explore OVX or IBN effects; whether modified fracture properties and greater brittleness are induced by long-term IBN alone remains to be seen. The modified mechanical properties in OVX+IBN are nonetheless consistent with an AF-like phenotype. Secondly, many human AF studies have centred upon microcracks.<sup>5,11</sup> In an attempt to pinpoint microcrack incidence in OVX+IBN

treated bones, we scanned at high resolution (1.3  $\mu\text{m}$  voxel as used previously;<sup>11</sup> tibia shaft regions showing distinct shape responses (25% to 45% and 60% to 85%)) to OVX+IBN, yet this failed to disclose microcracks. This aligns with studies reporting microdamage as extremely rare in mice.<sup>25</sup> Regardless, long-term bisphosphonates in humans precipitate an accumulation of microcracks that may diminish fracture strength.<sup>2,5,11</sup> Future work should explore if microdamage, stress fracture, or periosteal reactions are seen after OVX+IBN treatment. Thirdly, the necessity that biomechanical analyses require fresh, unfixed femora<sup>36</sup> precludes their use prior microCT evaluation, which itself requires samples are fixed to preserve integrity; lack of co-registration of mechanical and structural properties in a single bone is clearly a limitation. Finally, absence of murine intracortical remodelling makes direct comparison to human bone impossible.<sup>2,4,16</sup> Nonetheless, mice model their bones to maintain shape,<sup>15</sup> and long-term bisphosphonates effectively exert effects on the incumbent resorption. Mouse models have served an invaluable role in osteoporosis drug development and it is tempting to speculate that predictions regarding 'drug holiday' effectiveness may be made by monitoring of modelling related shape changes. Mice harbouring genetic AF predisposing polymorphisms or gene mutations can also be studied using our approach to resolve possible roles for genetics in AF aetiology.

Our data show that extended ibandronate treatment of previously ovariectomized mice modifies bone biomechanical properties to generate a stiffer, more brittle femur neck. OVX+IBN-treated mice also show tibia mass and shape changes consistent with bone straightening and deficient predicted strength. These findings make it tempting to speculate that OVX+IBN treatment in mice is an appropriate experimental setting for the study of AF pathophysiology.

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