

Response to Courtney et al.

Rutgers University has made this article freely available. Please share how this access benefits you.
Your story matters. <https://rucore.libraries.rutgers.edu/rutgers-lib/54734/story/>

This work is an **ACCEPTED MANUSCRIPT (AM)**

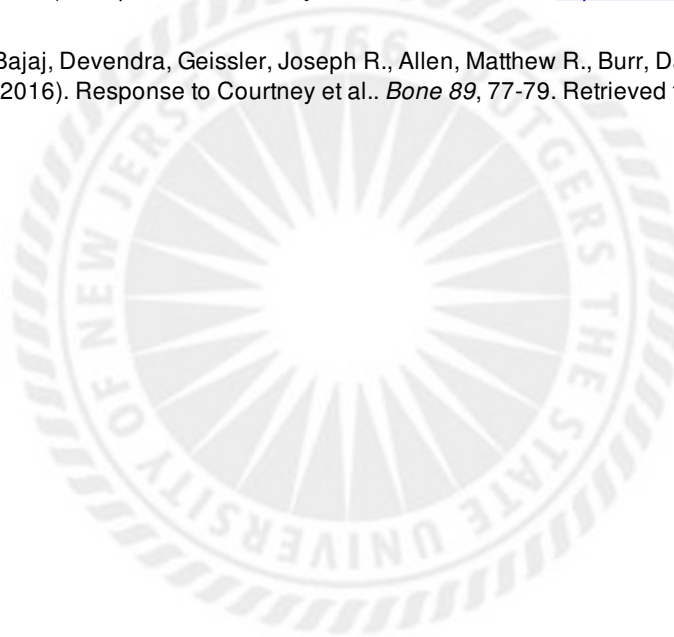
This is the author's manuscript for a work that has been accepted for publication. Changes resulting from the publishing process, such as copyediting, final layout, and pagination, may not be reflected in this document. The publisher takes permanent responsibility for the work. Content and layout follow publisher's submission requirements.

Citation for this version and the definitive version are shown below.

Citation to Publisher Bajaj, Devendra, Geissler, Joseph R., Allen, Matthew R., Burr, David B. & Fritton, J. Christopher.

Version: (2016). Response to Courtney et al.. *Bone* 89, 77-79. <http://dx.doi.org/10.1016/j.bone.2015.03.007>.

Citation to *this* Version: Bajaj, Devendra, Geissler, Joseph R., Allen, Matthew R., Burr, David B. & Fritton, J. Christopher. (2016). Response to Courtney et al.. *Bone* 89, 77-79. Retrieved from [doi:10.7282/T34F1TR0](https://doi.org/10.7282/T34F1TR0).



Terms of Use: Copyright for scholarly resources published in RUcore is retained by the copyright holder. By virtue of its appearance in this open access medium, you are free to use this resource, with proper attribution, in educational and other non-commercial settings. Other uses, such as reproduction or republication, may require the permission of the copyright holder.

Article begins on next page

Response to Courtney et al.

Devendra Bajaj, Ph.D.^a, Joseph R. Geissler, M.S.E.^{a,b}, Matthew R. Allen, Ph.D.^c,
David B. Burr, Ph.D.^c and J. Christopher Fritton, Ph.D.^{a,b}

^aDepartment of Orthopaedics, New Jersey Medical School, Rutgers University, 205 S. Orange Avenue, Newark, NJ 07103, USA, ^bDepartment of Biomedical Engineering, New Jersey Institute of Technology, 323 Martin Luther King, Jr. Boulevard, Newark, NJ 07102, USA, and ^cDepartment of Anatomy and Cell Biology, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, IN 46202, USA

E-mail addresses: devendrabajaj@gmail.com; geissljr@rutgers.edu;
matallen@iupui.edu; dburr@iupui.edu; chris.fritton@rutgers.edu

Funding sources: The National Institutes of Health (RR010601; AR007581; AR047838; AR062002; AR063351), the National Space Biomedical Research Institute (NASA contract NCC 9-58), the New Jersey Space Grant Consortium, Sigma Xi, and Merck (drug at no cost)

Address for correspondence:

J. Christopher Fritton, Ph.D.
Department of Orthopaedics
Rutgers, the State University of NJ
NJ Medical School, Cancer Center, G-1216
205 South Orange Avenue
Newark, NJ 07103, USA
Phone: (973) 972-2595
Fax: (973) 972-1875
Email: chris.fritton@rutgers.edu

References: 5

We refute the claims by Courtney et al., a group from Exponent, contracted by Merck, that our study was “ad hoc” in selecting certain data and that we did not fairly address limitations within the manuscript. However, we thank them for noting a mistake in our published manuscript regarding the labeling of specimens. Our conclusions remain the same.

As noted in the published manuscript our study was partially motivated by several studies on the mechanical behavior of short-term (1 year) and long-term (3 years) bisphosphonate-treated bones from beagle dogs that have demonstrated decreased toughness as estimated from a whole-bone, 3-point bending test of the rib. The last author (JCF) directed the first author, who was a post-doctoral fellow (DB), and the second author, a student (JRG), in this current project that was completed with the observers blinded to treatments. Three-year treatments of beagle dogs were completed by MRA and DBB after confirming skeletal maturity.

The group from Exponent has correctly noted that some of the specimens in the published manuscript were labeled as coming from the medial cortex of the dog rib when they were actually from the lateral cortex. JCF takes full responsibility for the mislabeling, and apologizes to the editor for not catching this mistake when completing the final draft of the manuscript. After un-blinding to complete the analyses it was noted that specimens excluded from the principal analysis, and found only in the manuscript supplement, were composed of specimens from the lateral cortex only. During the manuscript writing process these excluded specimens became known as “laterals.” Since specimens came from only two cortices, the other group mistakenly became “medials,” when in actuality some of the included specimens were from the lateral cortex. A Corrigendum has been filed with the correct labels of “included” and “excluded,” replacing “medial” and “lateral.”

The group from Exponent noticed this labeling mistake by analyzing the master’s thesis of JRG that is freely available for “fair use” (research and educational purposes) on the Internet. They base the majority of their arguments on this thesis and the appendices contained within. However, some descriptions of the analyses are missing or were misstated in the thesis. One specific error relates to a single sentence and figure within the Results chapter in which the student attempted to describe his initial observations of moduli within the groups. The description of the ultimate assignment of beams for inclusion in the primary analyses is missing from the Methods chapter of the student’s thesis. The failure to state that specimens were excluded if they did not undergo fatigue was an omission in the thesis that we regret, and for which we apologize.

We have corrected the specimen labeling error in the Corrigendum. Contrary to the claim by the group from Exponent, there was not “selective exclusion of some data points” in order to reach the conclusions in our manuscript. We also did not set a modulus criterion for exclusion of data as they imply (and may have been inferred from the student’s thesis). All beams that failed without reaching the secondary phase of fatigue by the middle of the complete cyclic test were excluded from the

primary analysis prior to un-blinding the investigators. *A priori* determination of which specimens will start to progress through the gradual phases of fatigue and which will fail quickly and/or without fatigue damage is not possible. Thus, discarding specimens that do not exhibit fatigue from cyclic tests is standard practice and is acknowledged as a limitation in our paper and in the study that the group from Exponent cites [1].

What is not standard practice in reports of fatigue tests of cortical bone tissue is inclusion of histomorphological measurements of the major microstructural components known to influence the biomechanical properties of that tissue (osteonal density, size and porosity) and inclusion of discarded data from the non-fatigued specimens in a publication. We included both in the supplement to the manuscript. These supplemental data demonstrate that the excluded beams had lower initial elastic moduli compared to the included beams. As we reported, the average initial modulus of beams was lower in the high-dose alendronate group compared to the vehicle-treated group (Fig. 2 in our manuscript). This is also true when initial modulus of all beams, including those from the low-dose animals, are included (-12%, ALN: 8.3 ± 1.7 , VEH: 9.4 ± 2.3 , $p < 0.05$).

Initial modulus is an intrinsic material property that largely influences the number of cycles to failure in the testing of bone tissue [2]. The lower initial modulus of the high-dose alendronate group suggests to us that tissue alterations, including damage accumulated over the 3 years of high-dose treatment, are responsible for the reduction in cycles to failure [3].

Modulus degradation (or “loss of modulus” as termed by the group from Exponent) occurs over a period lasting at most a couple of days and is one measure of fatigue during the cyclic testing of devitalized cortical bone tissue that we included in our manuscript for completeness. The study that the group from Exponent cites reported results from 29 *tensile* fatigue tests of femoral cortical bone from 4 humans and confirmed the interesting, implicit hypothesis that modulus degradation *rate* correlates with the number of fatigue cycles within this particular homogeneous, except for age, non-treated set of samples [1]. We concede that there was no statistically significant difference in absolute modulus degradation between groups included in our primary analysis. However, there are many differences between these studies, not limited to species, location, treatments and mechanical testing methods. The conclusion reached by the group from Exponent that a lack of statistically significant degradation differences in our study implies that the number of cycles to failure between groups should not be different requires a great many unstated assumptions and is not based on any existing published scientific literature.

Using further assumptions that the data from all of the beams should have been included in our fatigue analysis, the group from Exponent has plotted the “full data set, including data from the excluded specimens.” These data include beams that did not undergo fatigue. They analyzed the resulting very poorly fit curves to claim that

our paper has a false conclusion. In our opinion, their conclusion that specimens should not have been excluded is wrong because these specimens did not undergo fatigue. And, after un-blinding it would have been poor scientific procedure to exclude, in a *post hoc* manner, any specimens that did undergo fatigue.

We can understand the reluctance of the group from Exponent to agree with our conclusions as they have been retained by Merck's lawyers to provide expert witness to assist in introducing more doubt into the courtroom about the long-term effects of bisphosphonates on cortical bone. Our efforts have been in the laboratory where we expended a great deal of effort and care in this study to evaluate the effects of alendronate on the tissue-level mechanical and micro-structural properties of bone. We learned a great deal and the data in our paper support the conclusions reached for the cortical bone tissue beams of the 3-year treated dog ribs that we tested.

Part of the care we took in performing this study included the way we collected the cyclic mechanical data. Another false assumption by the group from Exponent, based on imprecise wording in the student's thesis, was that "data were recorded at major intervals of the logarithmic scale of the fatigue life plot" only [implied by the group from Exponent]. At least two cycles were fully recorded at intervals of at least every 1% of the major log divisions. This recording protocol provides for high fidelity in a fatigue test.

In our published manuscript several limitations were addressed by us within a lengthy (nearly full column) paragraph of the discussion. However, two observations made by the group from Exponent may deserve further discussion. First, in the primary analysis fewer specimens from the lateral cortex were included in vehicle control than in the drug-treated groups (Table S1). As we did not design our study to examine questions regarding the specific rib location from which beams were machined we are not able to definitively state the reasons that fewer vehicle control beams from the lateral cortex fatigued. We can speculate that if a different range of stress levels had been tested then at least two non-mutually exclusive possibilities might exist. One is that more or fewer beams from control might have undergone fatigue and been included in the primary analysis. Another is that more or fewer alendronate-treated beams might also have been included and again might have had a lower average initial modulus. There was also a trend for included beams to come from near the mid-shaft. In future studies even more attention should be paid to these location-specific issues as they likely play a role in fractures that are traditionally classified as non-traumatic. Again, our study was not designed to address this issue. The second observation is that if our inclusion/exclusion criteria had also included the requirement that groups be matched for initial modulus then no statistical differences between groups would likely be realized. This is most evident by examination of Fig. 3 in our manuscript where models adjusted for apparent strain amplitude demonstrated no statistically significant differences between groups. One could conceptualize this as normalizing applied stress by initial modulus. We addressed this issue and others in the

discussion.

The other assertions by the group from Exponent simply echo some of those other limitations addressed within the discussion. The main rehashed limitation is that bone is subject to creep under mechanical testing. We attempted to reduce the effects of creep with the inclusion/exclusion criteria that required fatigue to be well underway prior to the completion of testing. However, as indicated in the limitations, we cannot rule out that the long-term treatment had an effect on creep. As reported in our manuscript, tests were discontinued after 2.5 mm total deflection or 250,000 total cycles while the investigators were still blinded to group affiliation. Stopping tests is a common practice to protect the testing equipment from damage. We determined prior to any of the tests when to stop the tests. This was not data manipulation as the group from Exponent seems to suggest.

We do not assume that everyone reading this correspondence knows that Merck hired Exponent specifically to assist with legal defense against possible atypical femoral fracture civil-court cases. While the group from Exponent has disclosed that Merck contracted them, the “litigation” was not specifically stated in their disclosure statement. We should also be clear that one of our co-authors (DBB) is involved as an expert witness in this litigation on behalf of the plaintiffs. We do not assume that our data directly apply to possible atypical femoral fractures in humans and thus made no such conclusions. However, we would hope that our paper inspires a great deal more scientific research effort toward determining the effects of long-term treatments on the quality of bone tissue in order to further improve human health.

The data in our paper, supported by the previous published research cited within the paper, support the conclusions reached. Not included as references in our manuscript are two very recent publications, also supportive of our conclusions. One demonstrates reduced 4-point bending fatigue life of femoral shaft cortical bone tissue from sheep treated with clinical dosing of alendronate [4]. The other is a pooled analysis of previous whole-bone, mechanical tests of the mid-shaft of the dog rib. While the time-dependent, high-dose data had motivated our fatigue study, this new analysis demonstrates a significant trend for reduction in toughness, or energy to failure, associated with longer exposure to the lower alendronate dose from 3 months to 3 years, the longest term tested in the beagle dog model [5].

Devendra Bajaj, Ph.D.
Joseph R. Geissler, M.S.
Matthew R. Allen, Ph.D.
David B. Burr, Ph.D.
J. Christopher Fritton, Ph.D.
Email: chris.fritton@rutgers.edu

References

[1] Cotton JR, Winwood K, Zioupos P, Taylor M. Damage rate is a predictor of fatigue life and creep strain rate in tensile fatigue of human cortical bone samples. J

Biomech Eng 2005;127:213-9.

[2] Carter DR, Caler WE, Spengler DM, Frankel VH. Uniaxial fatigue of human cortical bone. The influence of tissue physical characteristics. J Biomech. 1981;14:461-70.

[3] Allen MR, Reinwald S, Burr DB. Alendronate reduces bone toughness of ribs without significantly increasing microdamage accumulation in dogs following 3 years of daily treatment. Calcif Tissue Int 2008;82:354-60.

[4] Brock GR, Chen JT, Ingraffea AR, MacLeay J, Pluhar GE, Boskey AL, van der Meulen MC. The effect of osteoporosis treatments on fatigue properties of cortical bone tissue. Bone Rep 2015;2:8-13.

[5] Burr DB, Liu Z, Allen MR. Duration-dependent effects of clinically relevant oral alendronate doses on cortical bone toughness in beagle dogs. Bone 2014;71:58-62.