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# Of mice, rats and men: Trabecular bone architecture in mammals scales to body mass with negative allometry

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ABSTRACT

Body mass (BM) in mammal species spans over six orders of magnitude. Although trabecular bone contributes to the mechanical properties of bones, we know much less about how trabecular bone scales with BM than about how cortical bone scales with BM. We therefore conducted a meta-analysis of the existing literature to test in rodents, humans and other mammals, predicted scaling properties between BM and several trabecular parameters: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity density (ConnD) and degree of anisotropy (DA). Our results show that BV/TV and DA are independent of BM and that Tb.N, Tb.Th and Tb.Sp scale with negative allometry relative to BM. Rodents appear to have relatively thicker and fewer trabeculae than humans, and we propose it that is due to a minimum thickness threshold "imposed" on mechanically functional trabeculae. Consequently, rodents (mice and rats) and humans demonstrate two distinct mechanisms to achieve variations in BV/TV. Although Tb.Th variation is the main contributing factor for differences in BV/TV in humans, Tb.N variation is the main contributing factor for differences in BV/TV in rodents. Our results also demonstrate no correlation between Tb.N and Tb.Th within each taxon (mice, rats and humans). Since rodents are a common animal model for research on bone biomechanics, the evidence that trabecular bone parameters scale and correlate differently in rodents than in humans suggests that care should be applied when extrapolating bone biomechanical results from small animals to large-bodied humans.



## 1. Introduction

Bone is a hierarchical composite material comprised in its lowest structural level of carbonated hydroxyapatite, collagen type I, several other non-collagenous proteins and water (Weiner and Wagner, 1998); in its highest structural level, bone is constructed of dense cortical and porous trabecular bone tissues (Weiner et al., 1999). Although all mammalian skeletal bones are practically identical as regard to their material components, their mechanical behavior differs both within and across species (Currey, 2003). While intra-species diversity is mainly due to heterogeneity in hydroxyapatite content and variations in cortical morphology and trabecular architecture (Currey, 2003; Fratzl and Weinkamer, 2007; Weiner and Wagner, 1998), differences across species are affected heavily by body mass (BM). It has been known since Galileo that forces act on the bones of small animals very differently than big animals, because bone strength scales to the power of two whereas mechanical loading scales to the power of three (Galilei, 1638). Consequently, as animals get bigger, their bones need to be more robust in order to withstand higher loads. Thus, whole bone scale their length and diameter relative to BM with close to isometry ($\propto BM^{0.33}$; i.e., the slope of the regression between the log of bone length or diameter and the log of BM is close to 0.33) (Alexander et al., 1979; Biewener, 1983; Steudel and Beattie, 1993). As trabecular bone tissue contributes to the mechanical properties of whole bones (Barak et al., 2008, 2010; Brodetti and Hirsch, 1956; Pennycuick, 1967; Rockoff et al., 1969; Rogers and LaBarbera, 1993; Werner et al., 1988), one would also expect trabecular bone properties such as trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) to scale relative to BM with close to isometry.

Despite the importance of scaling, few previous studies have looked at how trabecular bone parameters scale with body size across species. Mullender et al. (1996) compared bone volume

*Abbreviations:* BM, body mass; BS/TV, bone surface to total volume (bone volume fraction); BV/TV, bone volume to total volume (i.e., bone volume fraction); ConnD, connectivity density; DA, degree of anisotropy; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness.

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fraction (BV/TV), Tb.N, Tb.Th and Tb.Sp in the femoral head of five different mammal species (rat, rabbit, Rhesus monkey, pig and cow). Although they did not study the scaling of these trabecular bone properties with BM, and only looked at trabeculae in 2-D, the study does compare them across species. Their results showed that the range of Tb.Th and Tb.N values were relatively small between species (150–190 μm and 2.1–3.1 trabeculae/mm for Tb.Th and Tb.N respectively), except for rats which were significantly different (77 μm and 6.5 trabeculae/mm for Tb.Th and Tb.N respectively). Another 2-D study by Swartz et al. (1998) also found only a very weak relationship between Tb.Th and BM in the humeral and femoral head in a large sample of mammal species, but they did find that Tb.Th scaled close to isometry with BM within bats. They concluded that as body size increases the total number of trabeculae within a bone rather than Tb.Th increases in order to maintain adequate surface area for calcium homeostasis. A final recent study to note is Doube et al. (2011), which CT scanned the femoral head and lateral condyle of 72 terrestrial mammalian, 18 avian and one crocodilian species, thus including species with estimated body masses between 6 g and 3400 kg. This study found that, among mammals, BV/TV did not scale with body mass while Tb.Th and Tb.Sp increased with BM. They did not measure Tb.N; however they did show that connectivity density (which is highly correlated to Tb.N) decreased with BM.

Given the various unsolved questions regarding how trabecular bone scales with body mass, we designed a meta-analysis study to look at issues of scaling. The aims of our study are to address how trabecular bone scales with BM and to quantify the relationships between BV/TV, Tb.N. Tb.Th and Tb.Sp in different mammal species. Our first hypothesis, based on the few previous studies (Swartz et al., 1998; Doube et al., 2011), is that differences between mammal species in BV/TV and DA will not correlate to BM, while Tb.N, Tb.Th, Tb.Sp and Connectivity density (ConnD) will scale with negative allometry to BM. Secondly, we postulate that as BV/TV is determined by Tb.N and Tb.Th, the relation between BV/TV and Tb.N, and BV/TV and Tb.Th differs among mammals of varying size depending on the scaling of these trabecular bone properties with BM. Therefore, if our first hypothesis holds, Tb.N and Tb.Th may contribute differently to BV/TV in small vs. large mammals. Finally, we hypothesize that due to scaling, the relation between other trabecular bone properties also varies among mammals of different size (e.g. Tb.N vs. Tb.Th).

## 2. Materials and methods

### 2.1. Literature search and inclusion criteria

In order to identify relevant studies to include in the meta-analysis, systematic computerized searches were performed independently in Ovid and PubMed electronic databases for studies published prior to December 2010. The following search strings and keywords were used to search in the title and abstract of articles: [trabecula*], [cancellous], [(BV/TV) and (cancellous)], [(Tb.N) or (Tb.Th) or (Tb.Sp)]. Additional studies were identified by examining the reference lists of all articles identified. All on-line supplemental data was also inspected. Studies based on earlier data sets as well as duplicate experimental data sets were excluded.

Studies were included based on the following criteria: (1) the manuscripts were published in peer-reviewed journals in English, (2) the manuscript presented original data, (3) the study included a distinct control group of healthy individuals with no bone pathologies or signs of osteoporosis (only data from healthy and normal control groups were included in our study), (4) the study control group included only mature individuals and excluded juvenile or aged subjects, which have significantly different trabecular bone properties due to immature and growing skeletons or deterioration of the bone structure and osteopenia respectively, (5) in order to avoid subjectivity, several studies that compared multiple age, sex and treatment groups were also excluded. In such studies, there is no single objective way to pool the data, as these analyses often find complex patterns and significant differences between various groups, (6) measurement resolution was published and was sufficient to measure the trabecular properties of the species studied (Table 1S, online supplementary material), (7) the average values of at least BV/TV, Tb.N, Tb.Th, Tb.Sp, ConnD or DA were provided or could be calculated. In six papers the values were measured from the provided plot using Paint.NET v3.5.10, an image editing software (dotPDN LLC, Kirkland, WA, USA), which enables quantification of data points from ordinates by superimposing a pixel grid on an enlarged image (see Tables 1–3).

Applying these criteria yielded 51 papers on humans, 11 on nonhuman primates, 12 on rats, 9 on mice, 4 on cows, 3 on sheep, 2 on dogs, 2 on swine, 2 on rabbits, 1 paper on donkeys, 1 paper on horses and 1 paper on potoroos (a marsupial). Because some of these studies included more than one group eligible to participate in the meta-analysis – some papers contributed more than one data point. A complete summary of the studies included is given in the on-line supplementary material (Table 1S, online supplementary material).

As our study is a meta-analysis of the existing literature (including almost 100 manuscripts and spanning nearly two decades) it is important to explicate the vast amount of data (244 data points), the methods used in the various studies, their sensitivities and limitations. The majority of data points included in our meta-analysis were measured using a microCT (196 data points, 80.3% of all data points). The rest of data points were measured using microMRI (another 3D-measurment technique; 14 data points, 5.7% of all data points), histology (a 2D-measurment technique; 32 data

**Table 1**
Regression parameters for trabecular bone properties relationship with body mass.

| | | Regression slope | Regression intercept | *R* Value | *P* value (linear correlation) |
|---|---|---|---|---|---|
| BV/TV | All | −0.0092 | 1.41 | 0.045 | $P = 0.476$ |
| | Average | 0.0549 | 1.39 | 0.496 | $P = 0.121$ |
| DA | All | −0.27 | 0.32 | −0.133 | $P = 0.135$ |
| | Average | 0.033 | 0.23 | 0.568 | $P = 0.147$ |
| Tb.N | All | −0.146 | 0.42 | −0.748 | $P < 0.01$ |
| | Average | −0.106 | 0.46 | −0.810 | $P < 0.05$ |
| ConnD | All | −0.332 | 1.06 | −0.673 | $P < 0.01$ |
| | Average | −0.3 | 1.28 | −0.800 | $P < 0.05$ |
| Tb.Th | All | 0.137 | 1.99 | 0.659 | $P < 0.01$ |
| | Average | 0.124 | 1.98 | 0.756 | $P < 0.05$ |
| Tb.Sp | All | 0.137 | 2.51 | 0.649 | $P < 0.01$ |
| | Average | 0.082 | 2.42 | 0.567 | $P = 0.087$ |

For each trabecular bone parameter 2 regressions were calculated: (1) the regression for all existing data points (Fig. 1), and (2) the regression for the average value for each species. Using either method does not change the scaling relationship between each trabecular bone parameter and body mass.