Journal of Biomechanics ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Letter to the Editor

More thoughts on the relationship between apparent and material densities in bone

In a recent article of ours (Zioupos et al., 2008), we have shown that the apparent and material densities in bone show a bimodal 'boomerang' like behaviour with two branches: the first for cortical bone shows a positive relationship between the two densities, the second within cancellous bone shows an inverse relationship between these two quantities. In a recent letter, Baleani, Schileo and Taddei (Schileo et al., 2009) questioned the impact that this finding will have on CT-related studies and the universality of the aforementioned relationship.

We would like to address first the opening paragraph of Baleani and colleagues, which paraphrases our conclusions. We are afraid they misunderstood the main thrust of our paper. We did not conclude that an unequivocal delineation of cortical and cancellous bone can be done by CT scanning. In fact, we warned that apparent density, material density and mineral content are difficult to determine non-invasively for any one particular sample. It is ambiguous whether single sample is on this or that side of the threshold (the divide between cancellous and cortical bone areas). A series of diverse samples, however, can identify which samples lie on the upward or downward trends of the fundamental apparent vs. material density relationship we described in our paper (Zioupos et al., 2008). This is, because, the precise value for material or apparent densities and the threshold value will probably depend on the species and the skeletal location.

A notable example of a paper which corroborates our observations that mineral content shows a hyperbolic function with BV/TV (Zioupos et al., 2008; fig. 6, p.1967) is the work by Hernandez et al. (2001; fig. 1, p.75) who used data from Keller (1994). However, Hernandez et al. (2001), like most studies of the past, did not measure material (true) density, apparent density and mineral content as a set of three values at each point, instead they measured two and extrapolated the third. In spite of this their ash fraction (measured) vs. BV/TV (extrapolated) showed a dip in values for intermediate values of BV/TV of about 0.4 (for human bone) much like ours showed at about BV/TV = 0.6 (for elephant bone). The authors concluded then that there was a very poor linear correlation, but the scatter in the data was large and the pattern is clear.

Most past studies in this area suffered from the same two drawbacks: (1) one needs to actually measure material and apparent density and mineral content at each site and independently; and (2) most samples came from either cancellous or compact bone areas with few samples (no samples in case of Schileo et al., 2008) coming from the interface of intermediate BV/TV values. We have warned that although apparent density is a continuous function (between 0 and $\sim 2.3\,g/cm^3$), quite a few authors look into the behaviour of just two groups of tissue samples, which are of exclusively cancellous or cortical extremes. However, an examination of apparent density along the whole spectrum reveals two trends with material density depending onto whether one examines cancellous or cortical architecture. Testing groups of clearly different tissue misses the point of what happens at the interface. Apparent density does not fall in two bins for high value and low values. It shows a broad spectrum and it is a continuous variable.

As a word of caution we would also like to point out the difference between the ash fraction of Hernandez et al. (2001), our mineral fraction (Zioupos et al., 2008) and what a CT (or DXA) machine may in fact detect. The former are mass fractions of mineral mass per bone mass. A medical scanner, because of the principles of absorptiometry, is more likely to sense mass of mineral per volumetric unit of bone. Considering the 'boomerang' effect between material and apparent density and the hyperbolic function between ash fraction and BV/TV, the actual relationship between volumetric mineral content vs. BV/TV may in fact be inherently difficult to define by the scanners and in particularly in the interface (intermediate values of BV/TV). And then again this will also depend on the actual voxel size, with voxels at the micron scale behaving very differently from those at the millimetre scale.

Having said that there is always a further caveat, that it all depends on how one defines the BV/TV, and it is very likely that the value defined by histological methods would be slightly different from that defined by CT, or any other method. Let us agree to explicitly acknowledge the strengths and weaknesses of different methods before trying to compare histological/invasive methods with physical/non-invasive methods like CT. The latter depend critically on phantom-based calibrations and extrapolations with their associated errors and assumptions. We think it more likely that direct physical measurements and invasive methods are superior.

Baleani and colleagues comment that 'the literature shows mechanical similarity between cancellous and cortical bone'. Should we, therefore, assume that the mineral content is the same? Mechanical behaviour can derive from a number of factors, not just mineral content. Even if mineral content was constant (throughout the range of densities) architecture, orientation and micro-porosity would be able to derive a whole set of potentially different mechanical properties. This is a non-starter as an argument.

Do we need to corroborate the data with further 'independent' methods? Baleani and colleagues must mean 'alternative' methods, because the methods for measuring apparent material density and mineral content in our tests were independent of each other. Apparent density depends on cleaning and defatting, and measuring bone volume mechanically. Material density is based on suspension in fluid and the Archimedes principle. Mineral content is defined after ashing and the mass fractions.

doi:10.1016/j.jbiomech.2009.01.014

Baleani and colleagues question whether the pores in our measurements were indeed free of marrow. We mentioned the repeatability of our measurements, the use of a number of different experimenters and the different sets of tissue and all other such precautions. We perhaps forgot to state the particular attention we have taken to centrifuge the samples; in some cases in conjunction with methanol/chloroform; and how we went about perfecting these protocols over a number of years. As the material gets denser the pores may be more of close-packed cell geometry, but by then these are fewer in number and occupy lesser volume. We invite the authors to fill all the pores with a solid of density of about 1 and see what they make out of the sums. Even if we partly fill the pores with marrow above BV/TV of 0.5 that will only stretch the apparent density spectrum at its higher end, affecting its linearity and little else. If the boomerang effect is there, there is no way of escaping it.

As a concluding remark, we would like to mention one further implication of our findings. John Currey, on reading an early draft of our paper, commented: “you know there is at least one other physical variable that goes up and down from zero to zero. The bone area, that is the surface area around the pores; can be the two effects linked?” We think they may very well be. Indeed for a porosity of about 100% ($BV/TV = 0$) the surface area around the pores naturally goes to zero. When porosity drops to near zero ($BV/TV = 1$), so does the bone surface area. Inevitably between the two there ought to be a maximum leading to a parabolic, or boomerang-like behaviour between bone surface area and porosity (or BV/TV). If we think of the bone active surface area as the surface via which molecules transfer in and out of bone then the diffusion rate would be naturally benefited in some intermediate apparent density values where the area function is at its maximum value. It follows, therefore, that this diffusion-based metabolic effect would be lower for both very low and very high apparent density values and maximum somewhere in between. Higher remodelling would be then associated with lower material density and mineral content as shown in our paper. The two would indeed go hand-in-hand. We believe that this may provide more food for thought for bone researchers.

Conflict of interest

None.

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