

## COMMENTARIES

### Effects of Strontium Ranelate – Results Important But Presentation Muddled

A. Michael Parfitt

**Division of Endocrinology and Center for Osteoporosis and Metabolic Bone Diseases, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA**

**Commentary on:** Arlot ME, Jiang Y, Genant HK, Zhao J, Burt-Pichat B, Roux JP, Delmas PD, Meunier PJ. Histomorphometric and microCT analysis of bone biopsies from postmenopausal osteoporotic women treated with strontium ranelate. *J Bone Miner Res.* 2008 Feb;23(2):215-22.

Strontium ranelate (SR) appears able to prevent some fractures in postmenopausal osteoporosis, but its mode of action is unclear. Arlot *et al.* (1) recently reported that iliac biopsies in women given SR for one to five years showed that cancellous microarchitecture improved and cortical thickness increased. The data are of great interest but perpetuate some confusion about the relationships between two-dimensional (2D) and three-dimensional (3D) approaches to the study of architecture.

The complexity of cancellous bone architecture has been evident to anatomists, pathologists, radiologists, and bioengineers for more than a century, but until quite recently was largely ignored by physicians, clinical investigators and bone histomorphometrists. Arnold (2) first recognized the utility of regarding cancellous bone as an assemblage of interconnected plate-like or rod-like structural elements, which in the vertebral bodies are predominantly oriented either vertically or horizontally. He demonstrated how plates could be transformed to rods as initial perforations were progressively enlarged (3). Similar observations were made by Sissons (4;5) and Whitehouse (6;7) but their contributions were not heeded by those interested in osteoporosis.

For many years the only commonly used histologic index of cancellous bone structure was the proportion of bone tissue occupied by bone, referred to in the orthopedic

biomechanic literature as bone volume fraction, corresponding to bone volume/tissue volume (BV/TV) in the clinical literature; the transformation of a 2D area fraction to a 3D volume fraction was justified by the theorem of Delesse (8). Schenk was the first to apply stereology to the interpretation of bone histomorphometry (9). He showed how mean trabecular thickness could be estimated from the reciprocal of the bone surface to volume ratio (BS/BV), derived from the perimeter/area ratio  $\times \pi/4$  to correct for section obliquity.

About 25 years ago it occurred to me that using Schenk's method to calculate trabecular thickness and considering all cancellous bone present as aggregated into parallel plates, it was possible to calculate the number of plates in a given volume of cancellous bone tissue (10). The value is one-half of the specific surface (BS/TV) and represented the probability that a randomly superposed test line would intersect the profile of a structural element; "profile" is the term used by stereologists to denote the image in a 2D section corresponding to the 3D structure through which the section was made. We referred to the calculated results as mean trabecular plate density, thickness, and separation. The parallel plate model was a considerable oversimplification but was a great improvement over the previous practice of disregarding structure altogether. The indices could be derived from data already available so that no new measurements were needed, and

discriminated better than BV/TV alone between subjects with and without vertebral fracture (11). More importantly, the results indicated that during age-related cancellous bone loss, some structural elements were completely removed, and those remaining slowly became thinner, consistent with Arnold's proposal (3).

During the deliberations of the ASBMR histomorphometry nomenclature committee (12) we considered the terminology that I had coined to be unnecessarily cumbersome and recommended instead trabecular number, thickness and separation. The term "trabecular number", suggested as I recall by John Kanis, has become widely used, but several disadvantages are now evident. First, deleting the word "plate" has encouraged many people to forget the assumption on which the term was based (13). Second, the term trabecular number is subject to serious, although rarely recognized, ambiguity. Before explaining this, one must consider the concept of trabecular connectivity. This term has intuitive 3D significance when real examples of cancellous bone are inspected, but framing a rigorous definition has been quite challenging.

According to a Medline search, my former colleagues at Henry Ford Hospital and I were the first to refer to connectivity in the context of bone architecture (10;11). We had in mind a model of cancellous bone as a 3D rectangular lattice (14), corresponding to a late stage in Arnold's plate to rod transformation (3). Connectivity denoted the extent to which the struts of the lattice remained connected to each other; loss of connectivity resulted from transection of rods, as later illustrated by Liz Mosekilde (15). We did not then realize that the term "connectivity" already had a more precise and specialized meaning derived from topology – the maximum number of pathways that could be interrupted before the structure separated into two parts (16;17). Defined in this way, connectivity (C) is the 3D Euler number ( $N^{(3)}$ ) subtracted from 1 (16), and the number of trabeculae =  $C+1$  (17), =  $2 - N^{(3)}$ . Connectivity density ( $C/l^3$ ) is a reasonable predictor of trabecular

number as originally defined (16;17), but perforation of a trabecular plate will reduce "trabecular number" based on histomorphometry (because the probability of a test line intersection with a structural profile is reduced), but will increase "trabecular number" based on the topological definition (because the number of distinct pathways is increased)!

In the SR study (1) cancellous architecture was examined by microcomputed tomography ( $\mu$ CT). This method allows direct access to 3D structural information and can overcome all the limitations of the 2D parallel plate model (16). The original equipment was too large for widespread use, but improvements devised by R uegsegger (18) led to a desktop version (19), marketed by Scanco and used in the SR study (1). The authors cited two articles for the method of obtaining the reported structural indices, neither of which gave useful methodologic information; both referred to a recent book chapter (20) that relied heavily on the work of R uegsegger and his colleague (18;21-24). Trabecular thickness is defined for each point within bone as the diameter of the largest sphere that contains the point and lies completely within bone. The calculation provides not only the mean value but the frequency distribution of individual values (21). A similar procedure is used to calculate trabecular spacing (representing marrow cavity diameter), and the mean distance between the mid-axes of the trabeculae, of which the reciprocal is trabecular number, corresponding to the histologic rather than the topologic definition (22).

Regrettably, the authors failed to fully exploit the potential of their  $\mu$ CT method, since the reported values for trabecular number, thickness, and separation were derived from the means of multiple adjacent (stacked) slices, to each of which was applied the same calculations as for a single 2D histologic section. Because many slices were available, the precision of the mean values in each subject is greater, but the results in groups are similar to, and highly correlated with, those obtained by conventional histomorphometry, although

having a somewhat smaller coefficient of variation (23). Such data differ substantially from those obtained by true 3D calculations (22;23) and serve only to perpetuate the obsolete parallel plate model (24), which has now long outlived its usefulness (16).

Two genuine 3D indices were included – connectivity density, which showed no treatment effect, and structure model index, an estimate of the proportions of plate-like and rod-like structures (26), which was substantially lower (more plate-like) in the treated subjects (1). This is the most convincing evidence of micro-architectural improvement with SR, sufficient by itself to justify the authors' most important conclusion. Further application of  $\mu$ CT should concentrate on other 3D indices such as star volume (27) and degree of anisotropy (19;20), studying spatial heterogeneity (16), such as the much greater variability in trabecular thickness in the ilium compared to the vertebrae (21), and attempting to study the spatial relationship of remodeling processes to architecture (28). It would also be valuable to develop 3D analogs for interesting 2D indices such as fractal dimension (29), trabecular bone pattern factor (30) and node-strut analysis (31).

Although not the main theme of the current paper (1), the effects of SR on the bone remodeling process have also engendered considerable confusion. In various *in vitro* systems and animal models, SR has decreased some indices of bone resorption and increased some indices of bone formation (32). These observations have led to the claim that SR is able to induce "positive uncoupling" between resorption and formation (33), a claim that reflects a serious misconception. The term "coupling" refers only to the spatial and temporal relationships between resorption and formation and is a completely different concept from balance (34;35). The occurrence of bone formation only at locations where resorption has recently been completed is an inevitable result of the operation of bone remodeling as a replacement mechanism (35). If remodeling was uncoupled, there would be many

unfilled resorption cavities, and protuberances of new bone would be separated from old bone by a smooth and unscalloped cement line, but nothing like this has been reported. The data referred to could result from focal remodeling imbalance, due either to decreased resorption depth or increased wall thickness or both (35), but unfortunately the effect of SR on these measurements is unknown.

In some pathologic conditions, metaplastic woven bone may be made directly within the marrow, but with this exception, in the uninjured adult human skeleton new bone can only be formed in apposition to an existing bone surface (36). With this constraint, how could the structural changes observed in SR-treated patients have come about? Cortical thickness increased by 112  $\mu$ m in 1095 days, or about 0.102  $\mu$ m/d. One possible explanation is that SR reactivated periosteal modeling, as does intermittent PTH treatment (37), but the representative illustration suggests that the added bone was endocortical rather than periosteal (1). Even if activation frequency on the endocortical surface had been three times as high as on the cancellous surface at the time of biopsy (0.39/y) for the first two years of treatment, less than three cycles of remodeling could have been completed, and a positive remodeling balance of 40  $\mu$ m per cycle would be impossible. However, an anabolic effect of SR on the transitional zone (38) could have reversed age-related cancellization (39), reconverting the most peripheral cancellous bone back to cortical bone. Because more surface would be available, the necessary focal positive balance would be smaller, and because of the different geometry, less bone would be needed to increase cortical thickness by this means.

If new bone can be added only to an existing surface, how could cancellous architecture improve with no increase in trabecular thickness? Possibly a significant increase was missed because of the 2D rather than 3D interpretation of the data (22). But even so, how could "trabecular number" increase if new trabeculae can only be made in the growing skeleton (36)? The administration of

aluminum to dogs caused the outgrowth of new trabeculae from existing trabeculae, branching out into the marrow (40), but the histologic appearance is so bizarre that it could not have been overlooked. I think the paradox can be resolved by considering the geometry of plate perforation.

With the exception of the ends of transected rods, the cancellous surface is concave in curvature, but a perforation is like a porthole; at its periphery there is concave curvature parallel to the plate and convex curvature perpendicular to the plate, a combination referred to as a saddle surface. Bone remodeling activity occurs preferentially at such locations (15;28) and the conversion of a negative to a positive focal balance could eventually close a perforation without changing trabecular thickness. "Trabecular number" defined histologically would increase but "trabecular number" defined topologically would decrease! Pharmaceutical companies and clinical investigators need to pay more attention to the precise manner whereby an osteoporotic skeleton can be converted to a normal skeleton, but such understanding will never be achieved if the only question they ask about a new therapeutic agent is whether it "inhibits bone resorption" or "stimulates bone formation" (35) or both!

**Editorial Note from Juliet Compston:** This *Commentary* by Dr. Parfitt provides an excellent perspective on the measurement of cancellous bone microarchitecture and how this has been applied in a study of biopsies from phase III studies of strontium ranelate. It also considers, more briefly, the effects of strontium ranelate on bone remodeling. In addition, there are aspects of the design of this study that may affect the interpretation of the results. First, since the main microCT data came from unpaired biopsies, the differences between the placebo-treated and strontium ranelate-treated groups may reflect age-related deterioration in the placebo group, improvement in the treatment group or a combination of the two. Second, the number of paired biopsies (one placebo and 4 treated women) is too small to allow definite conclusions to be drawn from this part of the study. Third, in the 2D histomorphometric study, the inclusion in the control group both of baseline bone biopsies (from the placebo and treatment groups) and of placebo biopsies taken at different time points in the study complicates the interpretation of the results.

**Conflict of Interest:** None reported.

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