

Cancellous bone minimodeling-based formation: A Frost, Takahashi Legacy

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Abstract

The current dogma is that in adult human beings, remodeling creates nearly all the new cancellous bone tissue. However, Frost, Takahashi and colleagues hypothesized that minimodeling can go on in trabeculae throughout life. The current perspective will review the available reports on minimodeling-based formation to determine whether there is any support for his hypothesis. One: describe the methodology employed to characterize remodeling and minimodeling formation sites or packets, which restrict the analyses of these packets to a known age of the specimen. Two: present quantitative minimodeling data on cancellous bone of aging rats and transiliac bone biopsy of adult humans. Three: describe the occurrence and quantitation of mixed remodeling-minimodeling formation sites that could be misinterpreted as minimodeling sites. Fourth: present irrefutable evidence that bone anabolic agents initiate minimodeling-based formation sites. Fifth: discuss the mechanism of minimodeling-based formation may be the resumption of osteoblastic activity by bone lining cells to increase cancellous bone mass and trabecular connectivity. The findings of minimodeling is a rare activity in normal individuals, but may occur in a select population, and bone anabolic agents can initiate minimodeling-based formation are in support of Frost's hypothesis that minimodeling can continue throughout human life. Thus, another Frost, Takahashi legacy lives on.

Keywords: Bone Histomorphometry, Modeling, Minimodeling, Bone Anabolic Agents, Bone Lining Cells

The Frost, Takahashi and Colleagues Legacy

Frost, Takahashi and their colleagues define bone modeling as any biologic activity which shapes and sizes organs to provide optimal mechanical function (mechanical competence). Modeling can mean holding back growth in some places and increasing it in others to create and/or maintain functionally and purposefully shapes and sizes, usually for biomechanical reason. As an activity, bone formation and resorption in a specific bone site are independent and not coupled to each other. When modeling activities are induced on previously quiescent bone surfaces, either bone formation (A-F) or bone resorption (A-R) follows activation. As

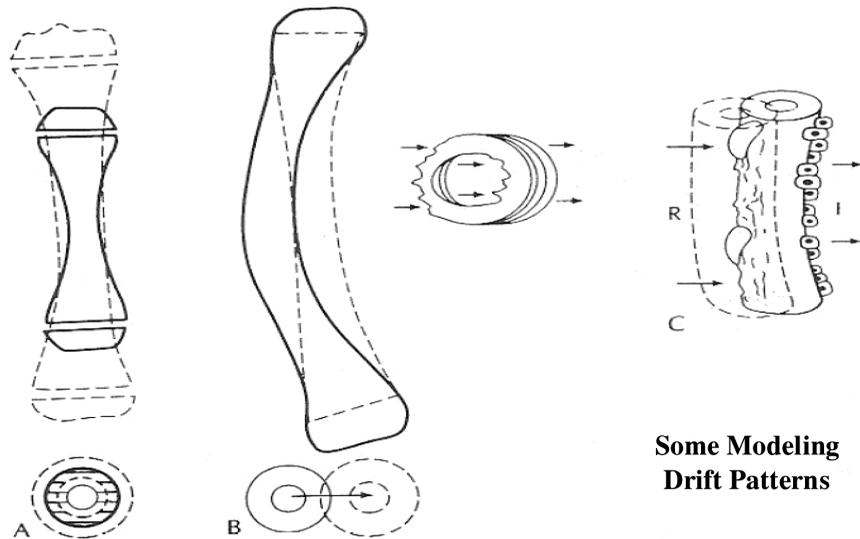
no osteoclastic bone resorption precedes bone formation in bone forming modeling sites, the cement lines are typically smooth. These cement lines are also called arrest lines because they are found during the temporary arrest of osteoblastic activity. In cortical bone the above kinds of modeling is defined as macromodeling, and for trabecula, minimodeling (Figure 1). Frost assumed that on trabeculae, the same modeling in miniature might provide adaptation to excessive strain, a process Dr. Frost termed minimodeling, because magnification is required to see it. In the rapidly growing skeleton, most of the cancellous bone-forming sites were minimodeling sites. There is a transition of bone turnover activity from modeling to remodeling in relation to a reduction in longitudinal growth with aging¹⁻⁶.

In 1964, Takahashi, Hattner, Epker and Frost¹ reported 96.7% of 5400 cement lines in 57 normal adults were scalloped. They further wrote, "with respect to the formation process occasionally "flows" beyond the perimeter of the preceding and underlying process. Thus if a section of that region, on the smooth part of the cement line, will be included in the section". The above observations were the first report that suggest such overfilling would increase mean wall thickness and overfilling of resorption cavities onto quies-

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Some Modeling Drift Patterns

Figure 1. Bone modeling patterns. **A** and **B**: example of macromodeling of cortical bone; **C**: example of minimodeling of cancellous bone. **A.** Pattern of bone surface drifts that maintain the gross shape of a growing long bone as it lengthens and increases in diameter. **B.** Modeling drifts to correct excessive longitudinal curvature of a long bone after a fracture. **C.** Minimodeling of trabecula showing the location of formation (F) and resorption (R) drifts. (From Frost HM. *Osteogenesis imperfecta. The set point proposal (a possible causative mechanism.) Clin Orthop Relat Res* 1987; 216:280-297 and printed with permission from Lippincott Williams & Wilkins).

cent surfaces, both resulting in a positive BMU balance, and minimodeling was a rare event in the cancellous bone in adult skeletons. Thus in the adult skeleton, remodeling creates nearly or all the new bone tissue⁷⁻¹⁰. Nevertheless in 1990, Frost hypothesized that mini-remodeling in trabeculae can go on throughout life⁵.

Comparison of remodeling and modeling parameters

Parfitt in 1983¹⁰ constructed a table comparing six important aspects of how modeling differs from remodeling in location, coupling, timing, extent, apposition rate and balance (Table 1, items 1-6). First (location), in modeling, resorption and formation occur on different bone surfaces. Second (coupling), in modeling, there is no coupling between resorption and formation to achieve changes in size and shape and is subject to independent regulation. Third (timing), the timing in modeling drift activity is continuous, whereas in remodeling each surface is subject to repeated cycles of resorption, formation and quiescence. Fourth (extent), in modeling surfaces the extent can be large. During growth, the entire periosteal and endocortical surface is the site of formation and resorption drift at all times. Fifth (apposition rate), in remodeling it is less than 1 $\mu\text{m}/\text{day}$ and is much slower than that with modeling of 5 $\mu\text{m}/\text{day}$ that varies with age. Sixth (balance), modeling produces a net gain in bone mass while remodeling produces either no change or net loss.

Over the years, I have updated Parfitt's original table to modify item 4 on extent for modeling and item 6 for balance for remodeling as well as add on 5 items involving cement line, surfaces, occurrence, function and minimum effective strain (MES) thresholds for humans (items 7-11, Table 1)¹⁴.

In item 4 (extent) under modeling, "variable" was substituted to cover the large surface involved in growing skeletons and small surface from of budding on trabecular surfaces by minimodeling in adult skeletons from select anabolic agents. Likewise in item 6 (balance), under remodeling, added was "gain" as in induced positive remodeling bone gain from anabolic agents. In addition, seventh (cement lines), the occurrence of smooth versus scalloped cement lines in modeling compared to remodeling units. Eighth (surfaces), remodeling activities are restricted to adjacent to marrow while at all surfaces with modeling, Ninth (occurrence), modeling is prominent during growth and ineffective in adults whereas remodeling occurs throughout life. Tenth (function), remodeling maintains bone mass and repairs microdamage whereas modeling changes shape and size for mechanical competence. Finally, eleventh (MES threshold), the estimated threshold for remodeling in humans is less than 1000 microstrain and to activate modeling is more than 2000 microstrain¹²⁻¹⁴ (Table 1).

In summary, the main characteristics of remodeling and modeling are as follows: Remodeling is the prevailing activity in the skeleton containing scalloped cement lines from A-R-F which maintains and repairs bone tissue; and modeling which is prominent during growth contains smooth cement lines, formation drift (A-F) and resorption drift (A-R) which sizes and shapes bone tissue.

Comparison of modeling and remodeling		
	Remodeling	Modeling
1. Location	Different surfaces	Spatially related
2. Coupling	A → R → F	A → F; A → R
3. Timing	Cyclical	Continuous
4. Extent	Small (<20%)	Variable*
5. Apposition rate	Slow (0.3-1.0 μm/day)	Fast (2-10 μm/day)
6. Balance	No change, net loss, or gain**	Net gain
7. Cement line	Scalloped	Smooth
8. Surfaces	Adjacent to marrow	All surfaces
9. Occurrence	Throughout life span	Prominent during growth; ineffective in adults
10. Function	Maintenance and repair	Skeletal adaptation of microdamage to mechanical usage (shape and size)
11. MES threshold	<1000 microstrain	>2000 microstrain

Table 1. Comparison of modeling and remodeling. MES=minimum effective strain; A=activation; R=resorption; F=formation. *variable: large (>90%) in growing bone and small in adult skeletons. ** gain from treatment with bone anabolic agents. (From: Parfitt AM. In: Recker RR (ed) *Bone Histomorphometry: Techniques and Interpretation*. CRC Press, Boca Raton, Florida; 1983:143-224¹⁰. From: Jee WSS In: Cowin S (ed) *Bone Mechanics Handbook*, second edition. CRC Press, Boca Raton, FL; 2001:1-25¹⁴).

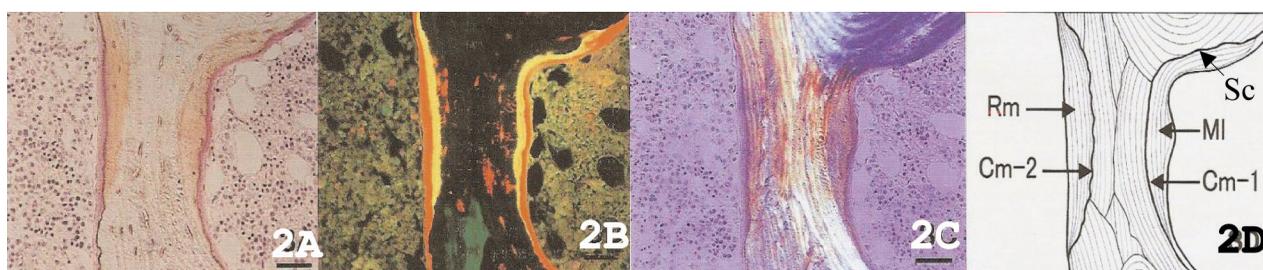


Figure 2. Remodeling formation site or packet on trabecula. Conventional (A), fluorescent (B), and polarized (C) light micrographs of a trabecula with minimodeling and remodeling in a transiliac bone biopsy specimen from a 74-year-old woman who underwent total hip arthroplasty for osteoarthritis of the hip (5 μm-thick undecalcified section; Villaneuva bone stain, scale bar=50 μm). (B) Fluorochrome labeling. (D) A schematic representation of C: Right packet: a mixed remodeling/modeling formation site (M1) with a scalloped cement line (Sc) followed by a smooth cement line (Cm-1) from the overlap of formation phase onto a quiescent surface. Left packet: a remodeling formation site (Rm), scalloped cement line (Cm-2), which interrupts surrounding collagen fibers. In their caption they failed to cited the right packet as a remodeling-based formation site but in reality it is a mixed remodeling-minimodeling packet. (Modified from Kobayashi et al. *Bone* 2003; 32:163-169¹⁶ and printed with permission from Elsevier).

Methods of characterizing remodeling and minimodeling formation sites

The technology to quantitate modeling remodeling sites involves the preparation of serial 5 μm thick undecalcified bone sections, multiple *in vivo* fluorochrome labeling, staining and classifying smooth and scalloped cement lines and polarized light microscopy to visualize collagen orientation¹⁵⁻¹⁷. The multiple fluorochrome labeling gives information helping to locate the position of the cement lines for a recent episode of bone formation. The labeling allows one to

restrict the analysis to bone structural units formed at a known age of the animal or human. To obtain excellent staining of cement lines, Erben recommends the surface staining technique with 1% Toluidine blue¹⁸, whereas Takahashi employed Villaneuva bone stain prior to methyl methacrylate embedding¹⁹. For each trabecular site actively forming bone, i.e., a labeled site, the corresponding cement line was analyzed using polarized light to visualize the orientation of collagen fibers. A remodeling site was defined with scalloped cement line with interrupted collagen fibers in the adjacent bone underlying the forming site, indicating previ-

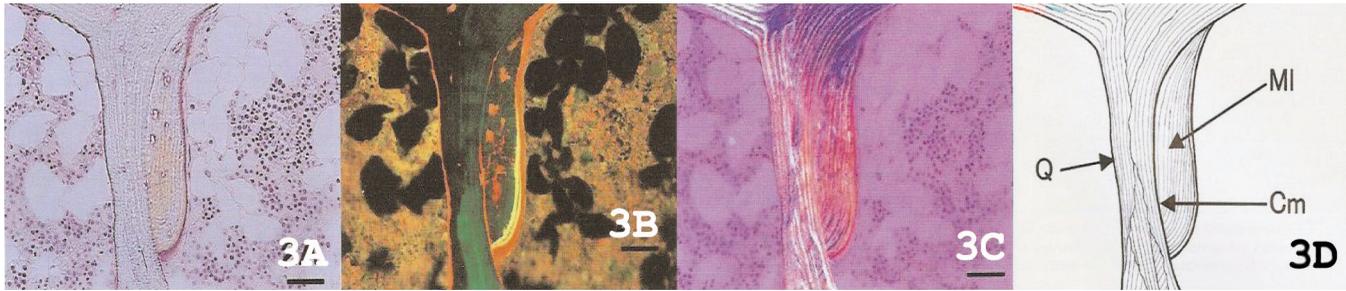


Figure 3. Minimodeling formation site or packet on trabecula. Conventional (A), fluorescent (B), and polarized (C) light micrographs of a trabecula with minimodeling in a transiliac bone biopsy specimen from a 69-year-old woman who underwent total hip arthroplasty for osteoarthritis of the hip (5 μm -thick undecalcified section; Villanueva bone stain; scale bar=50 μm). (B) Fluorochrome labeling at the modeling surface. (D) A schematic representation of C: M1, minimodeling; CM: smooth cement line which does not interrupt surrounding collagen fibers; and Q: quiescent surface. (Modified from Kobayashi et al. *Bone* 2003; 32:163-169¹⁶ and printed with permission from Elsevier).

ous resorption^{15-17,20-22} (Figures 2, 5A, 5B). Minimodeling site was defined with smooth cement line without interruption of surrounding collagen fibers^{15-17,20-22} (Figures 3, 5E, 5F).

Quantitative minimodeling data from aging rats

Erben¹⁵ did a classical remodeling/minimodeling study in his aim to elucidate whether bone resorption and bone formation are coupled in a manner typical of bone remodeling in the rat by analyzing cancellous bone in rats of different ages with a combination of *in vivo* fluorochrome labeling with cement line staining. In vertebral cancellous bone, remodeling was the main turnover activity at all age groups (3, 6, 9 and 12 months of age). In the proximal tibial metaphysis of 3-month-old rats they were classified as modeling, but in 12-month-olds remodeling was the dominant activity (Figure 4). There was reduced modeling and increased remodeling in relation to the reduction in longitudinal growth with aging. He concluded his study suggests that, similar to higher mammals, the prevailing activity in cancellous bone of aged rats is remodeling. Unfortunately, the study did not include older rats with sites containing closed growth plates.

Minimodeling data from adult human specimens

Minimodeling-based formation sites (packets) may be a rare phenomenon in normal and postmenopausal individuals. Takahashi et al.¹, reported that 3.3% of 5400 cement lines in cancellous bone from rib biopsy specimens from 57 normal adults. They commented that these smooth cement lines might have originated from overfilling of resorption cavities. However, in a study of iliac crest biopsy from patients undergoing total hip arthroplasty by Kobayashi et al.¹⁶ discovered 62% of 34 patients had packets with smooth cement lines. A comparison of specimens with and without minimodeling found that the presence of minimodeling packets was correlated with smaller physique of patient, accelerated mineralization, and

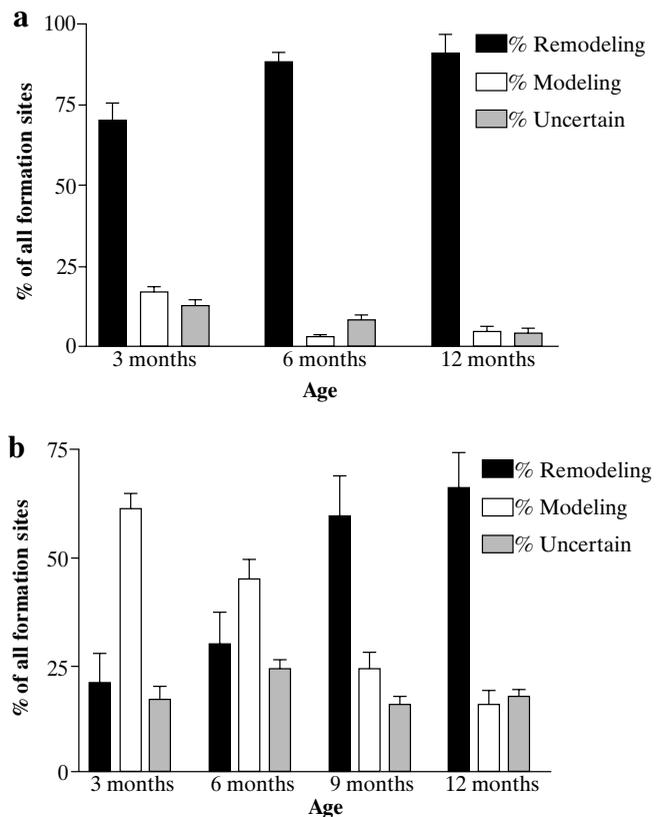


Figure 4. Modeling and remodeling activity in cancellous bone in aging rats. (a) Lumbar vertebral cancellous remodeling formation sites increased from $70.4 \pm 2.2\%$ (mean \pm SEM) in 3-month-old rats to $91 \pm 2.4\%$ in 12-month-old rats. The percentage of modeling sites decreased from $17.1 \pm 1.7\%$ at age 3 months to $4.67 \pm 1.84\%$ at age 12 months. (b) Proximal tibial metaphysis cancellous minimodeling site at 3 months of age was $61.6 \pm 3.6\%$ and $21.1 \pm 3.1\%$ as remodeling sites. In 12-month-old rats, $66.3 \pm 3.4\%$ was classified as remodeling and $16.0 \pm 3.1\%$ as modeling sites. (Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Erben RG. *Anat Rec* 1996; 246:39-46¹⁵).

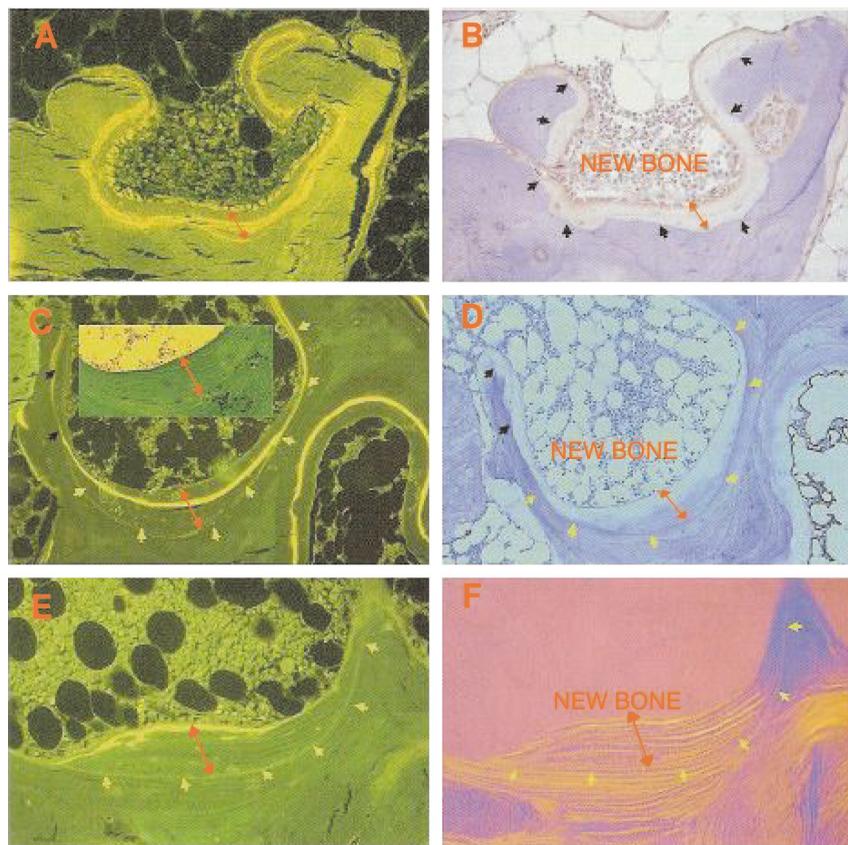


Figure 5. Teriparatide (1-34 hPTH) initiates remodeling (A, B), mixed remodeling-minimodeling (C, D) and minimodeling- (E, F) based formation sites or packets. (A, C, E) unstained fluorescent light images; (B, D) transmitted light for toluidine blue staining image specific for cement lines; and (F) polarized light image. Newly formed lamellar bone is indicated by double-ended arrow. (A, B) Remodeling-based packet with scalloped cement line (dark arrows) followed by a long smooth cement line (light arrows). (C, D) Mixed remodeling-minimodeling-based packet with short scalloped cement line (dark arrows) followed by a long smooth cement line (light arrows). The inset in C shows the presence of lamellar bone. (E, F) Minimodeling-based packet with smooth cement lines (light arrows) and with parallel collagen fiber orientation as the adjacent bone tissue indicating absence of previous bone resorption. (Adapted from *J Bone Miner Res* 2006; 21:855-854²⁰ with permission from the American Society for Bone Mineral Research).

higher bone turnover. Two recent studies dealing with iliac crest biopsy specimens reported no minimodeling packets. One was in a short-term study of the effect of teriparatide (1-34 hPTH) on transiliac bone biopsy specimens from 9 placebo-treated postmenopausal individuals²² and a second, a longer-term study with teriparatide that dealt with 55 baseline specimens, 22 were placebo-treated postmenopausal women²⁰, in which no typical minimodeling-based formation was found in these specimens.

Confounding factors in minimodeling formation sites: The mixed remodeling-modeling formation packet

As mentioned earlier, it was in 1964 that Takahashi et al.¹ suggested that the 3.3 percent minimodeling packet they observed may have actually originated from resorption cavities in which the formation phase overflowed onto quies-

cent bone surfaces. It was not until 2002 that Kobayashi et al.¹⁶, in their 2003 publication showed such a structure but failed to describe it as a mixed remodeling minimodeling packet possessing a short scalloped cement line followed by a longer smooth cement line (Figure 2D). In 2003, both Ma et al.^{17,19} and Lindsay et al.^{21,22}, described the overflowing of bone formation from remodeling sites after teriparatide treatment. Ma et al.^{17,19} named these 'mixed remodeling-minimodeling formation sites' or 'packets' (Figures 5C, 5D). Thus, such packets cannot be classified as modeling forming sites originating from quiescent bone surfaces, but as overflowing of remodeling sites overflowing on to quiescent bone surfaces or optimistically as mixed remodeling-minimodeling packets because it is not known whether such sites may have activated bone-lining cells to resume bone formation. Ma et al. also suggested that it is critical to analyze the minimodeling packets with longitudinal cutting orientation to avoid classifying the formation phase of a remodeling pack-

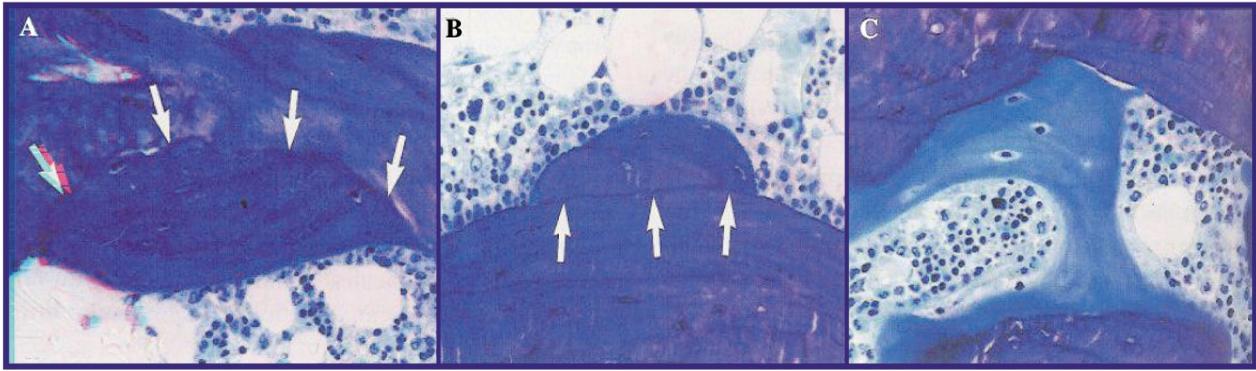


Figure 6. Increased trabecular width, minimodeling-based bone "bouton" or bud and reconnection of trabeculae in rat vertebral body cancellous bone. **A.** Chronic treatment with moderate to high doses of vitamin D analogs has positive effects on remodeling balance, resulting in overfilling of resorption cavities and a subsequent increase in trabecular width. The reversal line is marked with arrows. **B.** High doses of vitamin D analogs induce the formation of typical "bouton" by minimodeling with newly formed matrix on resting bone surfaces indicated by the smooth cement line (arrows). **C.** Under stimulation with very high doses of vitamin D analogs, adjacent bone spicules can become reconnected by excessive osteoblastic matrix formation. Note the accumulation of unmineralized osteoid. Toluidine blue surface stain for demonstration of cement lines. Original magnification x400. (Permission from Erben RG. *J Musculoskelet Neuronal Interact* 2001; 2:59-69³²)

et as a minimodeling packet. The other alternative is the tedious analyses of serial sections^{17,20}.

Another confounding factor would be that osteoclastic resorption might not leave behind a scalloped cement line. Osteoclasts could erode down to a trabecula and resorb along its lamellar plate without leaving a scalloped cement line. Thus, the assumption that smooth cement line implies minimodeling probably will be difficult to confirm²².

Bone anabolic agents initiate minimodeling-based formation

There is irrefutable evidence that bone anabolic agents (i.e., PTH, PGE₂ and vitamin D analogs) initiate minimodeling-based formation sites or packets. In animals, Zhou et al.²³ and Ciu et al.²⁴ reported daily PGE₂ treatment of 3 mg PGE₂/kg for 10 days in 20-month-old male rats initiated increased minimodeling packets in the cancellous bone of proximal tibial metaphysis and lumbar vertebral body by 4.3 and 2.3 folds, respectively, compared to controls. These minimodeling packet values may have been overestimated because some may be mixed-remodeling-minimodeling packets.

Short and long term teriparatide (1-34 hPTH) treatment on cancellous bone from human transiliac bone biopsy samples showed it initiated minimodeling-based formation sites (Figures 5E, 5F). Lindsay et al.²² quantitated minimodeling- and remodeling-based packets in postmenopausal women treated with teriparatide for 28 days to be 30.8% minimodeling based and 69.2% remodeling based formation. Again, the minimodeling formation sites may be overestimated because they may have originated from resorption sites. A 12-24 months 40 µg teriparatide study by Ma et al.¹⁷ found

minimodeling, mixed remodeling-minimodeling and remodeling-based formation of 3.8%, 3.9% and 92.3%, respectively, compared to placebo treated. Apparently, the dominant activity with longer teriparatide treatment is remodeling-based formation.

Target cells for minimodeling-based formation

Hodsman and Sheen²⁵ postulated that daily PTH could induce bone formation on quiescent surfaces or on minimodeling-based formation. The mechanism proposed is the resumption of bone formation by bone lining cells^{26,27}. Added evidence is PTH-induced increase in osteoblast number from the modulation of bone lining cells in a fatty marrow bone site, the caudal vertebral body³⁰. Because bone lining cells are abundant and lacking osteoprogenitor stem cells at skeletal sites with yellow marrow^{28,29}, the observation of PTH markedly increasing osteoblast surface in the caudal vertebra is consistent with the resumption of osteoblastic activity by bone lining cells³¹.

Increased trabecular connectivity with minimodeling-based formation

The animal findings suggest minimodeling-based formation would increase trabecular connectivity. Erben³² described adjacent spicules reconnected by excess osteoblastic formation with high doses of calcitriol, a vitamin D analog (Figure 6). Recent quantitative studies in rats treated with alfacalcidol, a vitamin D analog, found cancellous bone of the proximal tibial metaphysis and the lumbar vertebral body with increased trabecular connectivity of +52% and +160%, respectively. In addition, a strong correlation was

found between minimodeling-based bone bouton or buds and the increased connectivity (unpublished data).

The human transiliac bone biopsy data reported by Jiang et al.³³ reported teriparatide (1-34 hPTH) treatment of postmenopausal women with osteoporosis increased cancellous bone volume (teriparatide: +14%; placebo: -14%; $p=0.001$) and cancellous connectivity density (teriparatide: +19%; placebo: -14%; $p=0.034$). They concluded the increased bone volume and connectivity along with reduced marrow star volume and structure model index should improve biomechanical competence and are consistent with the observed reduction in vertebral and non-vertebral fractures.

Summary

In summary, although Kobayashi et al.¹⁶ reported an incidence of 62% minimodeling-based formation sites in transiliac bone biopsy specimens from 34 patients undergoing total hip arthroplasty, there are numerous studies indication that it is a rare event in normal individuals. These latter studies tend to support Frost's hypothesis that minimodeling-based formation can continue throughout human life⁵. However, more studies are needed in that Kobayashi et al.¹⁶ reported there was a subset of individuals with smaller physique, accelerated mineralization and higher bone turnover rate that only demonstrated minimodeling-based formation. There is irrefutable evidence that bone anabolic agents can initiate minimodeling-, mixed remodeling-, minimodeling and remodeling-based formations^{17,20-24}. Minimodeling activity was more prominent at early treatment²¹⁻²⁴. The mechanism of minimodeling-based formation is believed to be the resumption of osteoblastic activity by the bone lining cells^{25-27,30,31} leading to increased bone mass and trabecular connectivity^{32,33}. The unchallenged findings that anabolics can induce activity are in support of Frost's hypothesis that minimodeling can occur throughout life⁵. Thus, another legacy from Harold M. Frost and H.E. Takahashi lives on.

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