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Periosteum: Biology, regulation, and response to osteoporosis therapies

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1 Abstract

2 Periosteum contains osteogenic cells that regulate the outer shape of bone, and work in co-
3 ordination with inner cortical endosteum to regulate cortical thickness and the size and
4 position of a bone in space. Induction of periosteal expansion, especially at sites such as the
5 lumbar spine and femoral neck, reduces fracture risk by modifying bone dimensions to
6 increase bone strength. The cell and molecular mechanisms that selectively and specifically
7 activate periosteal expansion, as well as the mechanisms by which osteoporosis drugs
8 regulate periosteum remain poorly understood. We speculate that an alternate strategy to
9 protect human bones from fracture may be through targeting of the periosteum, either using
10 current or novel agents. In this review, we highlight current concepts of periosteal cell
11 biology, including their apparent differences from endosteal osteogenic cells, discuss the
12 limited data regarding how the periosteal surface is regulated by currently approved
13 osteoporosis drugs, and suggest one potential means through which targeting periosteum
14 may be achieved. Improving our understanding of mechanisms controlling periosteal
15 expansion will likely provide insights necessary to enhance current and develop novel
16 interventions to further reduce the risk of osteoporotic fractures.

17

1 **Introduction**

2 Osteoporosis drugs reduce fracture risk at clinically relevant sites, such as the femoral neck
3 and lumbar spine in postmenopausal women. Although increased bone mineral density
4 (BMD) contributes significantly to the reduced fracture risk [1], statistical analyses show the
5 protective effects of antiresorptive and anabolic drugs cannot be explained by increased
6 BMD alone [2, 3]. The effects of osteoporotic drugs on cortical bone surfaces, which
7 dictate bone geometry and thereby significantly influence overall strength, are less well
8 understood. Because femoral neck fractures initiate in cortical bone [4], and greater cortical
9 bone mass may explain the higher resistance to vertebral fracture in males [5, 6], cortical
10 bone biology clearly plays a major role in fracture prevention. Periosteal expansion of the
11 cortical shell significantly increases bone strength, independent of increases in areal bone
12 mineral density [7, 8]. This holds true even for bones composed predominantly of
13 trabecular bone, such as the femoral neck and lumbar vertebrae [9]. Gaining a better
14 understanding of how current osteoporosis drugs regulate cortical bone biology, especially
15 the preservation and expansion of periosteal surfaces, is critical to discovery of new
16 therapeutic regimens to reduce fractures.

17 Periosteum is a thin layer of osteogenic and fibroblastic cells in a well-developed
18 nerve and microvascular network, located along the periosteal cortex of cortical bone (Fig.
19 1). Because there are ligament and tendon muscle attachments, and fibrocartilage, on some
20 areas of the periosteal surface, the different physical environments to which periosteal cells
21 are exposed is quite unlike that of the more frequently studied endosteal cells which are
22 bathed in hematopoietic marrow. Compared to endosteal osteoblasts, periosteal osteoblasts
23 exhibit greater mechanosensitivity to strain [10], a lower threshold of responsiveness to
24 osteogenic compounds such as parathyroid hormone [11], higher levels of expression of

1 proteins such as periostin [12-14], and more estrogen alpha receptors [15]. These
2 differences in threshold sensitivity to physical, hormonal and mechanical stimuli may
3 underlie the differences in periosteal and endosteal surface responses to therapy [16]. More
4 extensive data are needed to fully characterize and understand the reasons for any difference
5 at the cellular level. Once this is accomplished, then periosteal cells can be targeted
6 therapeutically.

7 Our knowledge of the effects of approved osteoporosis drugs on cortical bone
8 biology is limited. Anti-resorptive and anabolic osteoporotic drugs may regulate periosteal
9 cells differently than endosteal cells. For mechanical reasons, periosteal stimulation may
10 provide better anti-fracture efficacy than agents that primarily target endosteal and
11 trabecular cell populations [17, 18]. We speculate that an alternate strategy to protect
12 human bones from fracture may be through targeting of the periosteum, either using current
13 or novel agents. In this review, we highlight current concepts of periosteal cell biology,
14 including their apparent differences from endosteal osteogenic cells, discuss the limited data
15 regarding how the periosteal surface is regulated by currently approved osteoporosis drugs,
16 and suggest one potential means through which targeting periosteum may be achieved.

17 **Periosteum anatomy**

18 Periosteum covers the external surfaces of most bones, to serve as a transitional region
19 between cortical bone and the overlying soft tissue or musculature. Long bones exhibit a
20 continuous periosteal surface, yet this surface may not be covered by an intact periosteum.
21 Periosteum is absent from articular surfaces, tendon insertions, or sesamoid bone surfaces
22 [19], and is present in locations at high risk for fracture, such as femoral neck, distal radius,
23 and vertebrae. The existence of periosteum at the femoral neck has been questioned; early
24 observational [20, 21] and histological [22] studies suggested the femoral neck lacked a

1 periosteal cambium layer. More comprehensive and recent histological studies show that
2 periosteum is present on human femoral neck surfaces [23-26], and in some cases, a thin
3 cambium layer with osteoblasts can be observed in discrete locations (Fig. 2) [26, 27].

4 Periosteum is composed of two distinct layers when viewed histologically (Fig. 1),
5 and of up to five distinctly different functional regions when dissociated enzymatically and
6 cultured [28-30]. Anatomically, the outer more “fibrous” layer of periosteum is composed of
7 fibroblasts, collagen, and elastin fibers [31] along with a distinctive nerve and microvascular
8 network [32, 33]. The inner “cambium” layer, positioned in direct contact with the bone
9 surface, is highly cellular. It contains adult mesenchymal progenitor cells, differentiated
10 osteogenic progenitor cells and osteoblasts [34], fibroblasts [35], as well as microvessels
11 [32] and sympathetic nerves [33]. Sympathetic nerve density is significantly higher
12 compared to the endosteum [36], but the relevance of this difference in terms of a
13 contribution to regulation of periosteum homeostasis and bone formation is not known.

14 Osteoblasts of the cambium layer are cuboidal in immature bone, becoming more
15 elongated [32] and fewer in number [37] with maturity. This reduction in osteoblast number
16 may contribute to the apparent atrophy and thinning of the cambium layer that occurs with
17 age [38]. Fibroblasts within the cambium layer are smaller and more isodiametric than
18 those in the outer fibrous layer, which have more typical (elongated) fibroblast
19 characteristics [35]. Periosteal fibroblast number and fibrous layer thickness decrease with
20 age [37], although atrophy of the fibrous layer is less than that of the cambium layer [32,
21 38]. Vessel density throughout the periosteum also declines with age [32], but retains the
22 capacity to increase when activated by mechanical loading or fracture repair [32]. These
23 age-induced changes may help explain why periosteal cells from older subjects fail to form
24 mineralized nodules in culture [39], and why periosteal bone formation rate [40], and

1 responsiveness to hormones and cytokines [41] declines with age. Whether such changes in
2 periosteal cells are also due to age-related changes in circulating hormones known to
3 influence the periosteum, such as growth hormone and sex steroids [42], deserves further
4 study.

5 Due to its high vascularity, the periosteum contains an abundance of endothelial
6 pericytes [43]. Pericytes are cells in physical contact with capillary endothelial cells, with
7 the ability to differentiate into numerous cell types, including osteoblasts, under appropriate
8 culture conditions [29, 44]. These cells may serve as a supplementary source of
9 osteoprogenitor cells [43] and may be more important in periosteal bone formation, due to
10 their greater abundance in periosteum [45], than in endosteal bone surface apposition [29].
11 Cultured pericytes mineralize in vitro [45] and synthesize the osteoblast marker, alkaline
12 phosphatase [45], as well as bone matrix proteins, including osteocalcin [32, 45],
13 osteonectin [32], osteopontin [32], and bone sialoprotein [32]. These cells form an
14 osteogenic tissue that mimics bone-derived tissue, both spatially and temporally [32] and
15 responds to osteogenic stimuli, such as BMP and parathyroid hormone [44]. A potential
16 role for pericytes as a source of osteoblasts in periosteum has not been investigated.

17 Site-specific differences in periosteum anatomy/activity clearly exist throughout the
18 skeleton. It is well know that the calvarial periosteum is uniquely regulated compared to
19 the axial skeleton (see below), and that cellular periosteum is sparse at the femoral neck
20 [24]. As the femoral neck increases periosteal dimensions with age [46, 47], the
21 consequences of having sparse periosteum are not clear. There are few studies that
22 specifically address the site-specific differences [48-50] yet clear differences (> 3 fold) in
23 periosteal bone formation rates exist among skeletal sites (Fig 3). Such varying rates
24 suggest periosteum anatomy/regulation may differ throughout the axial skeleton. Further

1 investigation of such site-specific differences is essential as it is possible that targeting the
2 periosteum may benefit only certain locations.

3 **Periosteal cells are unique from other osteogenic cell populations**

4 Most data detailing periosteal cell responses have been derived from cultures of calvarial-
5 derived cells, such as the MC3T3.E1 cell lines, primary calvarial cells or calvaria organ
6 cultures. Abuin and Triffitt [34] present an excellent review of the genetic regulation and
7 hormonal responsiveness of these cells. Although the embryonic mouse calvarial cell line
8 (MC3T3.E1) has been studied in great detail, its validity as a model for periosteal osteoblast
9 responses of appendicular and axial bones has not been adequately investigated. Calvarial
10 periosteum appears to be regulated differently from the periosteum of appendicular and
11 axial bones [51], and it is important to clarify whether MC3T3.E1 periosteal cells predict
12 generic periosteal responsiveness in culture, or are more representative of specific calvarial
13 periosteal responses. Furthermore, if this cell line is to be used as a prototype periosteal cell
14 model, it will be important for scientists in the field to reach consensus on the characteristics
15 of a reproducible phenotype, and standardized culture conditions, as these parameters
16 currently differ for the MC3T3.E1 cell line among laboratories [52].

17 Of studies using periosteal cells from appendicular or axial bones, few have directly
18 compared the response of periosteal and endosteal cells. Differences between periosteal and
19 endosteal cells are qualitative and quantitative, and range from patterns of growth in culture
20 [53] to the response to mechanical [10] and pharmacological stimuli [11]. Periosteal cells
21 divide more rapidly and mineralize in a more random pattern in vitro [53]. When exposed
22 to physiological levels of mechanical strain (3000 $\mu\epsilon$), periosteal cells increased proliferation
23 and PGE₂ production while osteoblasts of endosteal origin failed to respond [10]. Cultured
24 periosteal cells respond to PTH at a lower threshold, exhibiting a 7-fold increase in bone

1 matrix protein production compared to non-treated control cultures; cultured endosteal cells
2 inhibit bone matrix protein production when exposed to PTH [11]. Collectively, the data
3 support the idea that osteogenic cells display site-specific characteristics although the
4 limited studies make definitive conclusions difficult. If such differences are confirmed, it
5 will be essential to define how they translates to in vivo responses.

6 **Effect of approved osteoporosis drugs on periosteal expansion**

7 Current pharmacological interventions include anabolic and anti-resorptive agents. Both
8 modes of treatment reduce risk of osteoporotic bone fracture, in part by increasing bone
9 density. Anabolic agents, such as PTH, increase bone modeling [54] and remodeling [55].
10 Anti-resorptive agents, such as the bisphosphonates (e.g. alendronate, risedronate,
11 ibandronate, incadronate, or pamidronate), and estrogenic compounds (estrogen, raloxifene)
12 suppress bone remodeling through suppression of osteoclast resorption and increased
13 osteoclast apoptosis. The extent to which these various agents have surface-specific effects
14 and share common mechanistic pathways on the periosteal surface has not been studied in
15 depth.

16 Dual energy x-ray absorptiometry (DXA) is the most common form of skeletal
17 assessment in humans with differences in total cross-sectional area assumed to be related to
18 periosteal apposition. The limited resolution of DXA is well known [56], and may account
19 for the high variability among studies investigating pharmacological interventions in
20 humans. Despite limitations, the paucity of data on pharmacological effects on periosteal
21 bone in humans necessitates generalization to be drawn using such data.

22 Once daily parathyroid hormone (PTH) treatment increases cortical bone width
23 through preferential modeling on both periosteal and endosteal surfaces [56-60]. Cross
24 sectional clinical studies using DXA document significant increases in vertebrae [61] and

1 radius [62] cross sectional area following 12-18 months of PTH treatment in post-
2 menopausal women. Histological [54, 63-65] and microCT [65] data document PTH-
3 induced increases of cortical wall thickness. Femoral neck cortical bone volume increased
4 over 18 months with PTH treatment in post-menopausal women [66]. Data from paired
5 iliac crest biopsies suggests this to be the result of both endocortical and periosteal surface
6 formation [54]. Recent clinical trials document an attenuated BMD increases if anti-
7 resorptive agents are given prior to or simultaneously with PTH (1-34), suggesting
8 remodeling accounts for a significant portion of PTH-induced benefits [66-68]. Blunted
9 effects of PTH were noted in cancellous bone of animal previously treated with anti-
10 resorptive agents [69] whereas other animal studies show resorption is not necessary for
11 increased bone formation on cancellous bone [70, 71]; no such data exist on cortical bone.
12 More detailed studies that clearly show the surface- and time-specific effect of PTH have
13 been carried out in mice [60, 72]. Paradoxically, continuous exposure to PTH and
14 hyperparathyroidism in humans [59] and normal and genetically modified mice stimulates
15 periosteal surfaces, but fails to stimulate endocortical surfaces [73-79]. Even more
16 puzzling, constitutive activation of the PTH1 receptor in genetically modified mice inhibits
17 periosteal and endocortical bone formation, but stimulates trabecular bone formation [80].
18 Continuation of this work using genetically modified mouse models to elucidate the surface-
19 specific role of PTH is essential, as is more focused research on the periosteal surface
20 response to PTH in humans.

21 The anabolic effect of recombinant human growth hormone (rhGH) on periosteal
22 surfaces is well established in animals models [81], [82, 83] but remains unclear in humans
23 due to limited studies. Clinical trials using rhGH have documented increased cross sectional
24 area of the rib [84], lumbar vertebrae and femoral neck [85] using longitudinal biopsy and

1 DXA analyses. The interdependence of growth hormone and insulin-like growth factor I
2 (IGF-I) versus GH-independent effects of IGF-I make it difficult to independently assess the
3 contribution of each compound to periosteal apposition. IGF-I and its interaction with the
4 six known binding proteins influence periosteal geometry based on animal models [86, 87],
5 [88]. No studies to date have assessed IGF-I's influence on periosteal apposition in humans
6 although low-dose recombinant human IGF-I treatment for one month significantly
7 enhanced bone formation biomarkers in women [89]. When given at bone effective doses,
8 the side-effects of GH, and to a lesser degree IGF-I, have limited the advancement of human
9 studies to assess their use as osteoporosis therapies. Recently some investigators have
10 suggested such treatments could proceed with thorough oversight [90]. More research is
11 needed to understand how rhGH and its intermediaries regulate periosteal biology in
12 humans to determine if these are prototype agents to selectively stimulate periosteal
13 expansion and protect against osteoporotic fracture.

14 Few data document if bisphosphonate-induced reductions in bone remodeling impact
15 periosteal expansion. One year of high dose bisphosphonate treatment results in
16 significantly higher rib cross-sectional area (compared to controls) in dogs although
17 periosteal bone formation rate was not different at sacrifice [91]. This suggests the
18 increased rate of periosteal apposition was transient and occurred early during treatment.
19 Neither tibial diaphyseal periosteal perimeter of primates [92] nor iliac crest cortical width
20 of women were significantly altered [93] following prolonged bisphosphonate treatment.
21 Evidence suggests positive effects of bisphosphonates on osteoblasts in vitro [94-96], so the
22 selective effect of these drugs on periosteal modeling/remodeling should be assessed. The
23 periosteal surface displays evidence of bone resorption and therefore undergoes remodeling
24 [8, 97]. If the majority of apposition on the periosteal surface is remodeling driven, the

1 potential benefits of bisphosphonates on osteoblasts would not likely translate into new bone
2 formation although they could prevent some loss from this surface. If periosteal apposition
3 occurs via modeling processes, and if bisphosphonates suppress osteoblast apoptosis [98,
4 99], the benefits to bone strength by periosteal mechanisms could be significant.

5 Estrogen inhibits periosteal expansion while estrogen deficiency stimulates
6 periosteal expansion in animals [100-102]. Androgens stimulate periosteal apposition [103]
7 while androgen deficiency reduces periosteal apposition rates [101, 104, 105]. Periosteal
8 cells express both estrogen (alpha and beta) [15] and androgen receptors [106] as well as
9 numerous enzymes important for inter-conversion of sex steroids (i.e. aromatase, 5- α
10 reductase) [107, 108]. The estrogen receptor-alpha (ER α), is more highly expressed in
11 cortical bone [15], and appears a major regulator of periosteal apposition in males and
12 females. Mice lacking ER α receptors exhibit reduced periosteal diameter [109, 110] and an
13 attenuation of loading-induced periosteal apposition [111]. Osteoblasts lacking ER α do not
14 respond to strain in vitro [112]. Animals and cells lacking ER β are minimally affected with
15 respect to periosteal geometry and cellular activity. The absence of androgen receptors
16 (AR) abrogates testosterone-induced increases in periosteal bone formation [113] while
17 mice overexpressing AR exhibit increased periosteal formation rate [114]. These animal
18 and cell culture studies clearly document the influence of sex steroids on periosteal cell
19 activity, the effects of these hormones on human periosteal bone are less clear.

20 Pubertal changes in sex steroids account for the sexual dimorphism in human
21 periosteal geometry [115, 116]. How age-induced changes in exogenous sex steroid levels
22 influence periosteal expansion during the adult years, along with the effect of
23 pharmacological supplementation/replacement, are unclear. Reduced serum estrogen levels
24 occurring during menopause are associated with periosteal expansion and concomitant loss

1 of bone from endocortical and trabecular surfaces [7]. Estrogen replacement or hormone
2 replacement therapy increases periosteal apposition. Postmenopausal women taking
3 estrogen therapy for one year increased vertebral cross sectional area [61], and show a trend
4 toward increased iliac crest cortical width [117]. Cross sectional area of the femoral neck
5 and midshaft increased more in estrogen-treated postmenopausal women than in controls
6 [118]. There are no known data on periosteal changes with androgen treatment in humans.
7 Limited data suggest selective estrogen receptor modulators (SERMs) have little or no effect
8 on periosteal apposition in humans. Iliac crest biopsy data document a non-significant
9 increase in cortical bone width after two years of raloxifene treatment, compared to a
10 decrease in placebo treated subjects [119]. Clearly sex steroids and their interaction with
11 osteoblast receptors influence periosteal apposition. Elucidating the mechanisms of though
12 which these interactions regulate periosteal biology may lead to novel drug targets for
13 stimulating periosteal expansion.

14 Two important concepts influence the value of pharmacological stimulation of
15 periosteal apposition. First, we need to understand the comparative extent to which
16 periosteal apposition relies on modeling versus remodeling. Iliac crest data document both
17 modeling and remodeling on the periosteal surface in healthy women [97]. Differences in
18 the relative contribution of remodeling on cortical periosteal surfaces may determine the
19 relative benefit of anabolic and anti-resorptive treatments. Second, it is important to
20 understand the conditions under which periosteal apposition is related to endosteal
21 resorption. It is hypothesized that the loss of endocortical surface bone leads to higher stress
22 on the remaining bone, especially on the periosteal surface where stresses are highest in
23 bending, resulting in periosteal formation to normalize the stress [120]. Anti-resorptive
24 agents that reduce endocortical bone loss, and anabolic agents that increase endocortical

1 formation, could reduce the need for periosteal apposition if mechanical compensation is
2 absolute. It is likely that the magnitude of mechanical compensation depends on initial bone
3 size. Smaller bones exhibit more periosteal apposition in response to an equal absolute
4 amount of endocortical bone loss in larger bones [121]. Interactions between periosteal and
5 endosteal cortical bone surfaces, and the role that mechanical compensation plays in
6 periosteal expansion, necessitates more in-depth study.

7 **Possible mechanisms to target periosteal bone formation.**

8 In vivo studies of animals and post-menopausal women have revealed differences in the
9 osteogenic response on periosteal and endosteal surfaces, indicating a potential to
10 preferentially target the periosteal surface cells and increase bone circumference, thereby
11 reducing the risk of osteoporotic fracture. Selective targeting of the periosteum requires we
12 identify genes and proteins unique to periosteum, or present in greater concentrations in
13 periosteum. Recently, seven chromosomes that contain quantitative trait loci for periosteal
14 circumference in genetically altered mice were identified [122]. These data provide a
15 starting point from which to increase our understanding of the genetic control of periosteal
16 dimensions.

17 The relatively small quantity of periosteum at a given site other than calvaria, and
18 the difficulties in isolating relevant periosteal cells for such studies in animals and humans,
19 present hurdles that may be overcome by the use of technologies such as laser dissection
20 microscopy combined with molecular biology assays and tissue arrays, such as those used in
21 cancer. It is important to determine the relative extent to which animal models to predict
22 human periosteal cell responses. New data concerning periosteal adaptations in humans is
23 essential to improve our understanding of periosteal biology. More detailed cortical bone
24 analysis of iliac crest biopsies is necessary, beyond simply measuring cortical thickness. If

1 we are to gain a better understanding of how pharmacological interventions influence the
2 periosteal bone surface, more in-depth analyses should be undertaken including dynamic
3 bone formation assessment. The ability of the periosteum of the iliac crest to predict
4 periosteal responses at sites of greater osteoporotic fracture risk needs to be clarified.

5 To date, only one protein, periostin, is present in greater abundance in periosteum.
6 Periostin is localized predominantly in preosteoblasts, and secreted into the extracellular
7 matrix [12]. Periostin, originally termed OSF-2 [14], is highly expressed in the periosteum
8 cambium layer and in the mouse periosteal calvarial cell line, MC3T3.E1 during
9 proliferation [12]. Expression of periostin is increased 4-fold within three days of fracture
10 [123]. The transiently higher expression of periostin during osteoprogenitor proliferation
11 and abnormal osteoblast proliferation, and the decline in expression as differentiation
12 progresses, need to be better understood within the context of periosteal biology.
13 Expression of periostin is negatively regulated by 1,25-(OH)₂-D₃ [14] and positively
14 regulated by TGF-β [12, 14]. Through interaction with the promoter of a transcription
15 factor Twist, which is important for osteogenesis, periostin acts as a negative regulator for
16 osteoblast differentiation [13]. Further work is necessary to determine if the periostin-null
17 mouse can be used as a model to study periosteal adaptations.

18 **Conclusions**

19 This review takes a somewhat different approach than other recent reviews of
20 periosteal biology [8, 124-126] by focusing on the implications of the anatomical structure
21 of the periosteum and pointing out the limited data available from clinical trials with respect
22 to the effects of currently approved osteoporosis pharmaceuticals. Specifically, periosteal
23 cells appear to differ from endosteal cells; each cell population responds differently both
24 qualitatively and quantitatively to a wide variety of hormones and growth factors. We

1 suggest, after considering the limited published data of therapeutic interventions for
2 osteoporosis, that substantial work should be undertaken to assess how current drugs
3 influence periosteal cells. We speculate there are selective and specific drug targets within
4 the periosteum that can be activated independently of endocortical or trabecular surfaces.
5 Expanding the periosteal perimeter would represent a novel mechanism to dramatically
6 improve bone strength and reduce fracture risk, independent of the well-accepted effects of
7 increasing bone density.

8

1 **Figure captions**

2

3 **Figure 1.** Periosteal covering of the human femoral midshaft. Note the abundance of cells
4 (arrowheads) near the periosteal surface comprising the cambium layer. Section is from an
5 81 year old female cadaver stained with Massons trichrome. Original magnification x 400,
6 bar = 25 μ m.

7

8 **Figure 2.** Periosteal covering of the human femoral neck. Note the sparseness of cells
9 (arrowheads) near the periosteal surface as well as the abundant mineralized tissue (M) near
10 the periosteal surface. Section is from an 81 year old female cadaver stained with Massons
11 trichrome. Original magnification x 400, bar = 25 μ m.

12

13 **Figure 3.** Periosteal bone formation rates throughout the adult skeleton. Untreated adult
14 female cynomolgus monkeys (n=18) were injected with calcein three months apart and
15 formation rates were calculated at the radius mid-diaphysis, femoral neck, femoral mid-
16 diaphysis, 2nd lumbar vertebra, and humeral mid-diaphysis. Data presented as mean \pm SE.
17 Overall ANOVA p values = 0.04. See R. Brommage et al. J Clin Endocrinol Metab. 1999
18 for further study information.

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1 References

- 2 1. Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, LeBoff M,
3 Lewis CE, McGowan J, Neuner J, Pettinger M, Stefanick ML, Wactawski-Wende J,
4 Watts NB (2003) Effects of Estrogen Plus Progestin on Risk of Fracture and Bone
5 Mineral Density: The Women's Health Initiative Randomized Trial. *JAMA*
6 290:1729-1738
- 7 2. Cummings SR, Karpf DB, Harris F, Genant HK, Ensrud K, LaCroix AZ, Black DM
8 (2002) Improvement in spine bone density and reduction in risk of vertebral
9 fractures during treatment with antiresorptive drugs. *Am J Med* 112:281-289
- 10 3. Wasnich RD, Miller PD (2000) Antifracture Efficacy of Antiresorptive Agents Are
11 Related to Changes in Bone Density. *J Clin Endocrinol Metab* 85:231-236
- 12 4. Crabtree N, Loveridge N, Parker M, Rushton N, Power J, Bell KL, Beck TJ, Reeve J
13 (2001) Intracapsular hip fracture and the region-specific loss of cortical bone:
14 analysis by peripheral quantitative computed tomography. *J Bone Miner Res*
15 16:1318-1328
- 16 5. Duan Y, Seeman E, Turner CH (2001) The biomechanical basis of vertebral body
17 fragility in men and women. *J Bone Miner Res* 16:2276-2283
- 18 6. Kalender WA, Felsenberg D, Louis O, Lopez P, Klotz E, Osteaux M, Fraga J (1989)
19 Reference values for trabecular and cortical vertebral bone density in single and
20 dual-energy quantitative computed tomography. *Eur J Radiol* 9:75-80
- 21 7. Ahlborg H, Johnell O, Turner C, Rannevik G, Karlsson M (2003) Bone loss and
22 bone size after menopause. *N Engl J Med* 349:327-334
- 23 8. Orwoll E (2003) Toward an expanded understanding of the role of the periosteum in
24 skeletal health. *J Bone Miner Res* 18:949-954
- 25 9. Beck TJ, Ruff CB, Scott WW, Jr., Plato CC, Tobin JD, Quan CA (1992) Sex
26 differences in geometry of the femoral neck with aging: a structural analysis of bone
27 mineral data. *Calcif Tissue Int* 50:24-29
- 28 10. Jones DB, Nolte H, Scholubbers JG, Turner E, Veltel D (1991) Biochemical signal
29 transduction of mechanical strain in osteoblast-like cells. *Biomaterials* 12:101-110
- 30 11. Midura RJ, Su X, Morcuende JA, Tammi M, Tammi R (2003) Parathyroid hormone
31 rapidly stimulates hyaluronan synthesis by periosteal osteoblasts in the tibial
32 diaphysis of the growing rat. *J. Biol. Chem.*:M307567200
- 33 12. Horiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama
34 Y, Bonewald LF, Kudo A (1999) Identification and characterization of a novel
35 protein, periostin, with restricted expression to periosteum and periodontal ligament
36 and increased expression by transforming growth factor beta. *J Bone Miner Res*
37 14:1239-1249
- 38 13. Oshima A, Tanabe H, Yan T, Lowe GN, Glackin CA, Kudo A (2002) A novel
39 mechanism for the regulation of osteoblast differentiation: transcription of periostin,
40 a member of the fasciclin I family, is regulated by the bHLH transcription factor,
41 twist. *J Cell Biochem* 86:792-804
- 42 14. Takeshita S, Kikuno R, Tezuka K, Amann E (1993) Osteoblast-specific factor 2:
43 cloning of a putative bone adhesion protein with homology with the insect protein
44 fasciclin I. *Biochem J* 294 (Pt 1):271-278
- 45 15. Bord S, Horner A, Beavan S, Compston J (2001) Estrogen receptors alpha and beta
46 are differentially expressed in developing human bone. *J Clin Endocrinol Metab*

- 1 86:2309-2314
- 2 16. Epker BN, Frost HM (1965) A histological study of remodeling at the periosteal,
3 haversian canal, cortical endosteal, and trabecular endosteal surfaces in human rib.
4 Anat Rec 152:129-135
- 5 17. Frost HM (1973) Bone modeling and skeletal modeling errors. Thomas, Springfield,
6 IL
- 7 18. Ferretti JL, Frost HM, Gasser JA, High WB, Jee WS, Jerome C, Mosekilde L,
8 Thompson DD (1995) Perspectives on osteoporosis research: its focus and some
9 insights from a new paradigm. Calcif Tissue Int 57:399-404
- 10 19. Jee WS (2001) Integrated Bone Tissue Physiology: Anatomy and Physiology. In:
11 Cowin S (ed) Bone Mechanics Handbook. CRC Press, Boca Raton
- 12 20. Pankovich A (1975) Primary internal fixation of femoral neck fractures. Arch Surg
13 110:20-26
- 14 21. Phemister D (1939) The pathology of ununited fractures of the neck of the femur
15 with special reference to the head. J Bone Joint Surg Am 21:681-693
- 16 22. Banks H (1964) Healing of the femoral neck fracture. Proceedings of the Conference
17 on Aseptic Necrosis of the Femoral Head:465-482
- 18 23. Bagi CM, Wilkie D, Georgelos K, Williams D, Bertolini D (1997) Morphological
19 and structural characteristics of the proximal femur in human and rat. Bone 21:261-
20 267
- 21 24. Power J, Loveridge N, Rushton N, Parker M, Reeve J (2003) Evidence for bone
22 formation on the external "periosteal" surface of the femoral neck: a comparison of
23 intracapsular hip fracture cases and controls. Osteoporos Int 14:141-145
- 24 25. Shea JE, Hallows RK, Ricks S, Bloebaum RD (2002) Microvascularization of the
25 hypermineralized calcified fibrocartilage and cortical bone in the sheep proximal
26 femur. Anat Rec 268:365-370
- 27 26. Shea JE, Vajda EG, Bloebaum RD (2001) Evidence of a hypermineralised calcified
28 fibrocartilage on the human femoral neck and lesser trochanter. J Anat 198:153-162
- 29 27. Dixon T, Benjamin J, Lund P, Graham A, Krupinski E (2000) Femoral neck
30 buttressing: a radiographic and histologic analysis. Skeletal Radiol 29:587-592
- 31 28. Chyun Y, Kream B, Raisz L (1984) Cortisol decreases bone formation by inhibiting
32 periosteal cell proliferation. Endocrinology 114:477-480
- 33 29. Bianco P, Riminucci M, Gronthos S, Robey PG (2001) Bone marrow stromal stem
34 cells: nature, biology, and potential applications. Stem Cells 19:180-192
- 35 30. Raisz L (1984) Studies on bone formation and resorption in vitro. Hormone
36 Research 20:22-27
- 37 31. Hall B (ed) (1992) Bone Growth. CRC Press, Boca Raton
- 38 32. Ellender G, Feik SA, Carach BJ (1988) Periosteal structure and development in a rat
39 caudal vertebra. J Anat 158:173-187
- 40 33. Hohmann EL, Elde RP, Rysavy JA, Einzig S, Gebhard RL (1986) Innervation of
41 periosteum and bone by sympathetic vasoactive intestinal peptide-containing nerve
42 fibers. Science 232:868
- 43 34. Aubin J, Triffitt J (2002) Mesenchymal stem cells and osteoblast differentiation. In:
44 Bilezikian J, Raisz LG, Rodan GA (eds) Principles of Bone Biology. Academic
45 Press, San Diego
- 46 35. Squier C, Ghoneim S, Kremenak C (1990) Ultrastructure of the periosteum from
47 membrane bone. J Anat 171:233-239

- 1 36. Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD, Keyser CP,
2 Clohisy DR, Adams DJ, O'Leary P, Mantyh PW (2002) Origins of skeletal pain:
3 sensory and sympathetic innervation of the mouse femur. *Neuroscience* 113:155-166
- 4 37. Tonna EA, Cronkite EP (1963) The periosteum. Autoradiographic studies on cellular
5 proliferation and transformation utilizing tritiated thymidine. *Clin Orthop* 30:218-
6 233
- 7 38. O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS (2001) The chondrogenic potential
8 of periosteum decreases with age. *J Orthop Res* 19:95-103
- 9 39. Nakahara H, Goldberg VM, Caplan AI (1991) Culture-expanded human periosteal-
10 derived cells exhibit osteochondral potential in vivo. *J Orthop Res* 9:465-476
- 11 40. Epker BN, Frost HM (1966) Periosteal appositional bone growth from age two to
12 age seventy in man. A tetracycline evaluation. *Anat Rec* 154:573-577
- 13 41. Pfeilschifter J, Diel I, Pilz U, Brunotte K, Naumann A, Ziegler R (1993) Mitogenic
14 responsiveness of human bone cells in vitro to hormones and growth factors
15 decreases with age. *J Bone Miner Res* 8:707-717
- 16 42. Kim BT, Mosekilde L, Duan Y, Zhang XZ, Tornvig L, Thomsen JS, Seeman E
17 (2003) The structural and hormonal basis of sex differences in peak appendicular
18 bone strength in rats. *J Bone Miner Res* 18:150-155
- 19 43. Diaz-Flores L, Gutierrez R, Lopez-Alonso A, Gonzalez R, Varela H (1992)
20 Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. *Clin*
21 *Orthop*:280-286
- 22 44. Reilly TM, Seldes R, Luchetti W, Brighton CT (1998) Similarities in the phenotypic
23 expression of pericytes and bone cells. *Clin Orthop*:95-103
- 24 45. Brighton CT, Lorich DG, Kupcha R, Reilly TM, Jones AR, Woodbury RA, 2nd
25 (1992) The pericyte as a possible osteoblast progenitor cell. *Clin Orthop*:287-299
- 26 46. Beck TJ, Ruff CB, Bissessur K (1993) Age-related changes in female femoral neck
27 geometry: implications for bone strength. *Calcif Tissue Int* 53 Suppl 1:S41-46
- 28 47. Ruff CB, Hayes WC (1982) Subperiosteal expansion and cortical remodeling of the
29 human femur and tibia with aging. *Science* 217:945-948
- 30 48. Anderson C, Danylchuk K (1978) Bone-remodeling rates of the beagle: a
31 comparison between different sites on the same rib. *Am J Vet Res* 39:1763-1765
- 32 49. Miller SC, Bowman BM, Miller MA, Bagi CM (1991) Calcium absorption and
33 osseous organ-, tissue-, and envelope-specific changes following ovariectomy in
34 rats. *Bone* 12:439-446
- 35 50. Sheng M, Baylink D, Beamer W, Donahue L, Rosen C, Lau K, Wergedal J (1999)
36 Histomorphometric studies show that bone formation and bone mineral apposition
37 rates are greater in C3H/HeJ (high-density) than C57BL/6J (low-density) mice
38 during growth. *Bone* 25:421-429
- 39 51. Hock JM, Kream BE, Raisz LG (1982) Autoradiographic study of the effect of 1,25-
40 dihydroxyvitamin D3 on bone matrix synthesis in vitamin D replete rats. *Calcif*
41 *Tissue Int* 34:347-351
- 42 52. Leis HJ, Hulla W, Gruber R, Huber E, Zach D, Gleispach H, Windischhofer W
43 (1997) Phenotypic heterogeneity of osteoblast-like MC3T3-E1 cells: changes of
44 bradykinin-induced prostaglandin E2 production during osteoblast maturation. *J*
45 *Bone Miner Res* 12:541-551
- 46 53. Solchaga LA, Cassiede P, Caplan AI (1998) Different response to osteo-inductive
47 agents in bone marrow- and periosteum-derived cell preparations. *Acta Orthop*

- 1 Scand 69:426-432
- 2 54. Dempster D, Zhou H, Cosman F, Nieves J, Adachi J, Frahar L, Watson P, Lindsay
3 B, Hodsman A (2001) PTH treatment directly stimulates bone formation in
4 cancellous and cortical bone in humans. *J Bone Miner Res* 16 (Supp 1):S179
- 5 55. Mashiba T, Burr DB, Turner CH, Sato M, Cain RL, Hock JM (2001) Effects of
6 human parathyroid hormone (1-34), LY333334, on bone mass, remodeling, and
7 mechanical properties of cortical bone during the first remodeling cycle in rabbits.
8 *Bone* 28:538-547
- 9 56. Bolotin H, Sievanen H (2001) Inaccuracies inherent in dual-energy x-ray
10 absorptiometry in vivo bone mineral density can seriously mislead
11 diagnostic/prognostic interpretations of patient-specific bone fragility. *J Bone Miner
12 Res* 16:799
- 13 57. Burr D, Hirano T, Turner C, Hotchkiss C, Brommage R, Hock J (2001)
14 Intermittently administered human parathyroid hormone(1-34) treatment increases
15 intracortical bone turnover and porosity without reducing bone strength in the
16 humerus of ovariectomized cynomolgus monkeys. *Journal of Bone and Mineral
17 Research* 16:157-165
- 18 58. Parfitt A (1994) Osteonal and hemi-osteonal remodeling: the spatial and temporal
19 framework for signal traffic in adult human bone. *J. Cellular Biochemistry* 55:273-
20 286
- 21 59. Parfitt AM (2002) Parathyroid hormone and periosteal bone expansion. *J Bone
22 Miner Res* 17:1741-1743
- 23 60. Mohan S, Kutilek S, Zhang C, Shen H, Y. K, Srivastava A (2000) Comparison of
24 bone formation responses to parathyroid hormone (1-34), (1-31), and (2-34) in mice.
25 *Bone* 27:471-478
- 26 61. Rehman Q, Lang TF, Arnaud CD, Modin GW, Lane NE (2003) Daily treatment with
27 parathyroid hormone is associated with an increase in vertebral cross-sectional area
28 in postmenopausal women with glucocorticoid-induced osteoporosis. *Osteoporos Int*
29 14:77-81
- 30 62. Zanchetta JR, Bogado CE, Ferretti JL, Wang O, Wilson MG, Sato M, Gaich GA,
31 Dalsky GP, Myers SL (2003) Effects of teriparatide [recombinant human
32 parathyroid hormone (1-34)] on cortical bone in postmenopausal women with
33 osteoporosis. *J Bone Miner Res* 18:539-543
- 34 63. Dempster DW, Cosman F, Kurland ES, Zhou H, Nieves J, Woelfert L, Shane E,
35 Plavetic K, Muller R, Bilezikian J, Lindsay R (2001) Effects of daily treatment with
36 parathyroid hormone on bone microarchitecture and turnover in patients with
37 osteoporosis: a paired biopsy study. *J Bone Miner Res* 16:1846-1853
- 38 64. Hodsman AB, Kisiel M, Adachi JD, Fraher LJ, Watson PH (2000)
39 Histomorphometric evidence for increased bone turnover without change in cortical
40 thickness or porosity after 2 years of cyclical hPTH(1-34) therapy in women with
41 severe osteoporosis. *Bone* 27:311-318
- 42 65. Jiang Y, Zhao J, Mitlak B, Wang O, Genant H, Eriksen E (2003) Recombinant
43 human parathyroid hormone (1-34) [teriparatide] improves both cortical and
44 cancellous bone structure. *J Bone Miner Res* 18:1932-1941
- 45 66. Black DM, Greenspan SL, Ensrud KE, Palermo L, McGowan JA, Lang TF, Garnero
46 P, Bouxsein ML, Bilezikian JP, Rosen CJ (2003) The effects of parathyroid hormone
47 and alendronate alone or in combination in postmenopausal osteoporosis. *N Engl J*

- 1 Med 349:1207-1215
- 2 67. Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H, Neer RM (2003) The
3 effects of parathyroid hormone, alendronate, or both in men with osteoporosis. *N*
4 *Engl J Med* 349:1216-1226
- 5 68. Ettinger B, San Martin J, Crans G, Pavo I (2004) Differential effects of teriparatide
6 on BMD after treatment with raloxifene or alendronate. *J Bone Miner Res* 19:745-
7 751
- 8 69. Delmas PD, Vergnaud P, Arlot ME, Pastoureau P, Meunier PJ, Nilssen MH (1995)
9 The anabolic effect of human PTH (1-34) on bone formation is blunted when bone
10 resorption is inhibited by the bisphosphonate tiludronate--is activated resorption a
11 prerequisite for the in vivo effect of PTH on formation in a remodeling system?
12 *Bone* 16:603-610
- 13 70. Ma YL, Bryant HU, Zeng Q, Schmidt A, Hoover J, Cole HW, Yao W, Jee WS, Sato
14 M (2003) New bone formation with teriparatide [human parathyroid hormone-(1-
15 34)] is not retarded by long-term pretreatment with alendronate, estrogen, or
16 raloxifene in ovariectomized rats. *Endocrinology* 144:2008-2015
- 17 71. Hock JM, Hummert JR, Boyce R, Fonseca J, Raisz LG (1989) Resorption is not
18 essential for the stimulation of bone growth by hPTH-(1-34) in rats in vivo. *J Bone*
19 *Miner Res* 4:449-458
- 20 72. Hock JM (2000) Discrimination among osteoblasts? Parathyroid hormone analog
21 may reveal site-specific differences in mice. *Bone* 27:467-469
- 22 73. Morinaga T, Nakagawa N, Yasuda H, Tsuda E, Higashio K (1998) Cloning and
23 characterization of the gene encoding human osteoprotegerin/osteoclastogenesis-
24 inhibitory factor. *Eur J Biochem* 254:685-691
- 25 74. Christiansen P, Steiniche T, Brixen K, Hesse I, Melsen F, Heickendorff L,
26 Mosekilde L (1999) Primary hyperparathyroidism: short-term changes in bone
27 remodeling and bone mineral density following parathyroidectomy. *Bone* 25:237-
28 244
- 29 75. Christiansen P, Steiniche T, Mosekilde L, Hesse I, Melsen F (1990) Primary
30 hyperparathyroidism: changes in trabecular bone remodeling following surgical
31 treatment--evaluated by histomorphometric methods. *Bone* 11:75-79
- 32 76. Christiansen P, Steiniche T, Vesterby A, Mosekilde L, Hesse I, Melsen F (1992)
33 Primary hyperparathyroidism: iliac crest trabecular bone volume, structure,
34 remodeling, and balance evaluated by histomorphometric methods. *Bone* 13:41-49
- 35 77. Dobnig H, Turner RT (1997) The Effects of Programmed Administration of Human
36 Parathyroid Hormone Fragment (1-34) on Bone Histomorphometry and Serum
37 Chemistry in Rats. *Endocrinology* 138:4607-4612
- 38 78. Jaeger P, Jones W, Kashgarian M, Baron R, Clemens T, Segre G, Hayslett J (1987)
39 Animal model of primary hyperparathyroidism. *Am J Physiol* 252:E790-798
- 40 79. Imanishi Y, Hosokawa Y, Yoshimoto K, Schipani E, Mallya S, Papanikolaou A,
41 Kifor O, Tokura T, Sablosky M, Ledgard F, Gronowicz G, Wang TC, Schmidt EV,
42 Hall C, Brown EM, Bronson R, Arnold A (2001) Primary hyperparathyroidism
43 caused by parathyroid-targeted overexpression of cyclin D1 in transgenic mice. *J.*
44 *Clin. Invest.* 107:1093-1102
- 45 80. Calvi LM, Sims NA, Hunzelman JL, Knight MC, Giovannetti A, Saxton JM,
46 Kronenberg HM, Baron R, Schipani E (2001) Activated parathyroid
47 hormone/parathyroid hormone-related protein receptor in osteoblastic cells

- 1 differentially affects cortical and trabecular bone. *J Clin Invest* 107:277-286
- 2 81. Harris WH, Heaney RP, Jowsey J, Cockin J, Akins C, Graham J, Weinberg EH
3 (1972) Growth hormone: the effect on skeletal renewal in the adult dog. I.
4 Morphometric studies. *Calcif Tissue Res* 10:1-13
- 5 82. Andreassen TT, Melsen F, Oxlund H (1996) The influence of growth hormone on
6 cancellous and cortical bone of the vertebral body in aged rats. *J Bone Miner Res*
7 11:1094-1102
- 8 83. Andreassen TT, Oxlund H (2000) The influence of combined parathyroid hormone
9 and growth hormone treatment on cortical bone in aged ovariectomized rats. *J Bone*
10 *Miner Res* 15:2266-2275
- 11 84. Kruse HP, Kuhlencordt F (1975) On an attempt to treat primary and secondary
12 osteoporosis with human growth hormone. *Horm Metab Res* 7:488-491
- 13 85. Landin-Wilhelmsen K, Nilsson A, Bosaeus I, Bengtsson BA (2003) Growth
14 hormone increases bone mineral content in postmenopausal osteoporosis: a
15 randomized placebo-controlled trial. *J Bone Miner Res* 18:393-405
- 16 86. Mohan S, Richman C, Guo R, Amaar Y, Donahue LR, Wergedal J, Baylink DJ
17 (2003) Insulin-Like Growth Factor Regulates Peak Bone Mineral Density in Mice by
18 Both Growth Hormone-Dependent and -Independent Mechanisms. *Endocrinology*
19 144:929-936
- 20 87. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi GT,
21 Setser J, Frystyk J, Boisclair YR, LeRoith D (2002) Circulating levels of IGF-1
22 directly regulate bone growth and density. *J Clin Invest* 110:771-781
- 23 88. Tobias JH, Chow JW, Chambers TJ (1992) Opposite effects of insulin-like growth
24 factor-I on the formation of trabecular and cortical bone in adult female rats.
25 *Endocrinology* 131:2387-2392
- 26 89. Ghiron LJ, Thompson JL, Holloway L, Hintz RL, Butterfield GE, Hoffman AR,
27 Marcus R (1995) Effects of recombinant insulin-like growth factor-I and growth
28 hormone on bone turnover in elderly women. *J Bone Miner Res* 10:1844-1852
- 29 90. Rosen CJ, Wuster C (2003) Growth hormone rising: did we quit too quickly? *J Bone*
30 *Miner Res* 18:406-409
- 31 91. Mashiba T, Hirano T, Turner CH, Forwood MR, Johnston CC, Burr DB (2000)
32 Suppressed bone turnover by bisphosphonates increases microdamage accumulation
33 and reduces some biomechanical properties in dog rib. *J Bone Miner Res* 15:613-
34 620
- 35 92. Itoh F, Kojima M, Furihata-Komatsu H, Aoyagi S, Kusama H, Komatsu H,
36 Nakamura T (2002) Reductions in bone mass, structure, and strength in axial and
37 appendicular skeletons associated with increased turnover after ovariectomy in
38 mature cynomolgus monkeys and preventive effects of clodronate. *J Bone Miner Res*
39 17:534-543
- 40 93. Dufresne T, Chmielewski P, Manhart M, Johnson T, Borah B (2003) Risedronate
41 Preserves Bone Architecture in Early Postmenopausal Women In 1 Year as
42 Measured by Three-Dimensional Microcomputed Tomography. *Calcif Tissue Int*
43 73:423-432
- 44 94. Giuliani N, Pedrazzoni M, Negri G, Passeri G, Impicciatore M, Girasole G (1998)
45 Bisphosphonates stimulate formation of osteoblast precursors and mineralized
46 nodules in murine and human bone marrow cultures in vitro and promote early
47 osteoblastogenesis in young and aged mice in vivo. *Bone* 22:455-461

- 1 95. Goziotis A, Sukhu B, Torontali M, Dowhaniuk M, Tenenbaum HC (1995) Effects of
2 bisphosphonates APD and HEBP on bone metabolism in vitro. *Bone* 16:317S-327S
- 3 96. Im GI, Qureshi SA, Kenney J, Rubash HE, Shanbhag AS (2004) Osteoblast
4 proliferation and maturation by bisphosphonates. *Biomaterials* 25:4105-4115
- 5 97. Balena R, Shih MS, Parfitt AM (1992) Bone resorption and formation on the
6 periosteal envelope of the ilium: a histomorphometric study in healthy women. *J*
7 *Bone Miner Res* 7:1475-1482
- 8 98. Plotkin LI, Manolagas SC, Bellido T (2002) Transduction of cell survival signals by
9 connexin-43 hemichannels. *J Biol Chem* 277:8648-8657
- 10 99. Plotkin LI, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T
11 (1999) Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and
12 calcitonin. *J Clin Invest* 104:1363-1374
- 13 100. Turner RT, Riggs BL, Spelsberg TC (1994) Skeletal effects of estrogen. *Endocr Rev*
14 15:275-300
- 15 101. Turner RT, Hannon KS, Demers LM, Buchanan J, Bell NH (1989) Differential
16 effects of gonadal function on bone histomorphometry in male and female rats. *J*
17 *Bone Miner Res* 4:557-563
- 18 102. Wakley GK, Evans GL, Turner RT (1997) Short-term effects of high dose estrogen
19 on tibiae of growing male rats. *Calcif Tissue Int* 60:37-42
- 20 103. Coxam V, Bowman BM, Mecham M, Roth CM, Miller MA, Miller SC (1996)
21 Effects of dihydrotestosterone alone and combined with estrogen on bone mineral
22 density, bone growth, and formation rates in ovariectomized rats. *Bone* 19:107-114
- 23 104. Gunness M, Orwoll E (1995) Early induction of alterations in cancellous and cortical
24 bone histology after orchietomy in mature rats. *J Bone Miner Res* 10:1735-1744
- 25 105. Lea C, Kendall N, Flanagan AM (1996) Casodex (a nonsteroidal antiandrogen)
26 reduces cancellous, endosteal, and periosteal bone formation in estrogen-replete
27 female rats. *Calcif Tissue Int* 58:268-272
- 28 106. Kasperk C, Helmboldt A, Borcsok I, Heuthe S, Cloos O, Niethard F, Ziegler R
29 (1997) Skeletal site-dependent expression of the androgen receptor in human
30 osteoblastic cell populations. *Calcif Tissue Int* 61:464-473
- 31 107. Shozu M, Simpson ER (1998) Aromatase expression of human osteoblast-like cells.
32 *Mol Cell Endocrinol* 139:117-129
- 33 108. Turner RT, Bleiberg B, Colvard DS, Keeting PE, Evans G, Spelsberg TC (1990)
34 Failure of isolated rat tibial periosteal cells to 5 alpha reduce testosterone to 5 alpha-
35 dihydrotestosterone. *J Bone Miner Res* 5:775-779
- 36 109. Vidal O, Lindberg MK, Hollberg K, Baylink DJ, Andersson G, Lubahn DB, Mohan
37 S, Gustafsson J-A, Ohlsson C (2000) Estrogen receptor specificity in the regulation
38 of skeletal growth and maturation in male mice. *Proceedings of the National*
39 *Academy of Sciences* 97:5474-5479
- 40 110. Sims NA, Dupont S, Krust A, Clement-Lacroix P, Minet D, Resche-Rigon M,
41 Gaillard-Kelly M, Baron R (2002) Deletion of estrogen receptors reveals a
42 regulatory role for estrogen receptors-[beta] in bone remodeling in females but not in
43 males. *Bone* 30:18-25
- 44 111. Lee K, Jessop H, Suswillo R, Zaman G, Lanyon L (2003) Endocrinology: bone
45 adaptation requires oestrogen receptor-alpha. *Nature* 424:389
- 46 112. Jessop H, Suswillow R, Rawlinson S, Zaman G, Lee K, Das-Gupta V, Pitsillides A,
47 Lanyon L (2004) Osteoblast-like cells from estrogen receptor [alpha] knockout mice

- 1 have deficient responses to mechanical strain. *J Bone Miner Res* 19:938-946
- 2 113. Vandendput L, Swinnen J, SBoonen S, Van Herck E, Erben R, Bouillion R,
3 Vanderschueren D Role of the androgen receptor in skeletal homeostasis: the
4 androgen-resistant testicular feminized male mouse model. *J Bone Miner Res*
5 Published online May 10, 2004; doi: 10.1359/JBMR.040505
- 6 114. Wiren K, Zhang X, Toombs A, Kasparcova V, Gentile M, Harada S, Jepsen K
7 Targeted overexpression of androgen receptor in osteoblasts: unexpected complex
8 bone phenotype in growing animals. *Endocrinology* Published online May 6, 2004;
9 doi: 10.1210/en.2003-1016
- 10 115. Seeman E (2003) The structural and biomechanical basis of the gain and loss of bone
11 strength in women and men. *Endocrinol Metab Clin North Am* 32:25-38
- 12 116. Seeman E (2001) Clinical review 137: Sexual dimorphism in skeletal size, density,
13 and strength. *J Clin Endocrinol Metab* 86:4576-4584
- 14 117. Vedi S, Bell KL, Loveridge N, Garrahan N, Purdie DW, Compston JE (2003) The
15 effects of hormone replacement therapy on cortical bone in postmenopausal women.
16 A histomorphometric study. *Bone* 33:330-334
- 17 118. Beck TJ, Stone KL, Oreskovic TL, Hochberg MC, Nevitt MC, Genant HK,
18 Cummings SR (2001) Effects of current and discontinued estrogen replacement
19 therapy on hip structural geometry: the study of osteoporotic fractures. *J Bone Miner*
20 *Res* 16:2103-2110
- 21 119. Ott SM, Oleksik A, Lu Y, Harper K, Lips P (2002) Bone histomorphometric and
22 biochemical marker results of a 2-year placebo-controlled trial of raloxifene in
23 postmenopausal women. *J Bone Miner Res* 17:341-348
- 24 120. Lazenby RA (1990) Continuing periosteal apposition. I: Documentation, hypotheses,
25 and interpretation. *Am J Phys Anthropol* 82:451-472
- 26 121. Seeman E, Duan Y (2004) Measurement Issues in Periosteal Apposition. *J Bone*
27 *Miner Res* 19:691
- 28 122. Masinde GL, Wergedal J, Davidson H, Mohan S, Li R, Li X, Baylink DJ (2003)
29 Quantitative trait loci for periosteal circumference (PC): identification of single loci
30 and epistatic effects in F2 MRL/SJL mice. *Bone* 32:554-560
- 31 123. Nakazawa T, Nakajima A, Seki N, Okawa A, Kato M, Moriya H, Amizuka N,
32 Einhorn TA, Yamazaki M (2004) Gene expression of periostin in the early stage of
33 fracture healing detected by cDNA microarray analysis. *J Orthop Res* 22:520-525
- 34 124. Seeman E (2003) Periosteal bone formation--a neglected determinant of bone
35 strength. *N Engl J Med* 349:320-323
- 36 125. Seeman E (2003) Reduced bone formation and increased bone resorption: rational
37 targets for the treatment of osteoporosis. *Osteoporos Int* 14 Suppl 3:S2-8
- 38 126. Seeman E (2002) An exercise in geometry. *J Bone Miner Res* 17:373-380
- 39

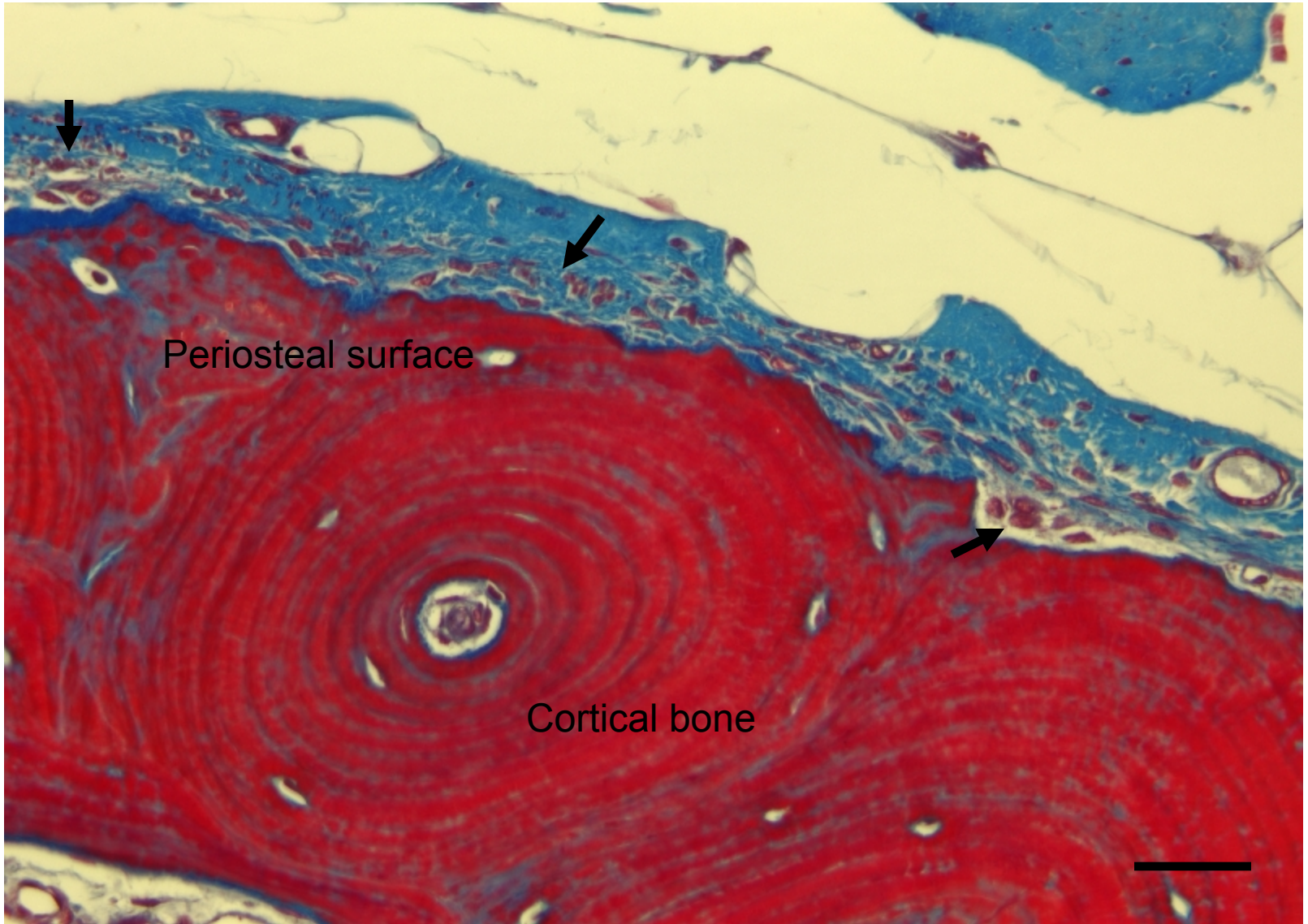


Figure 1

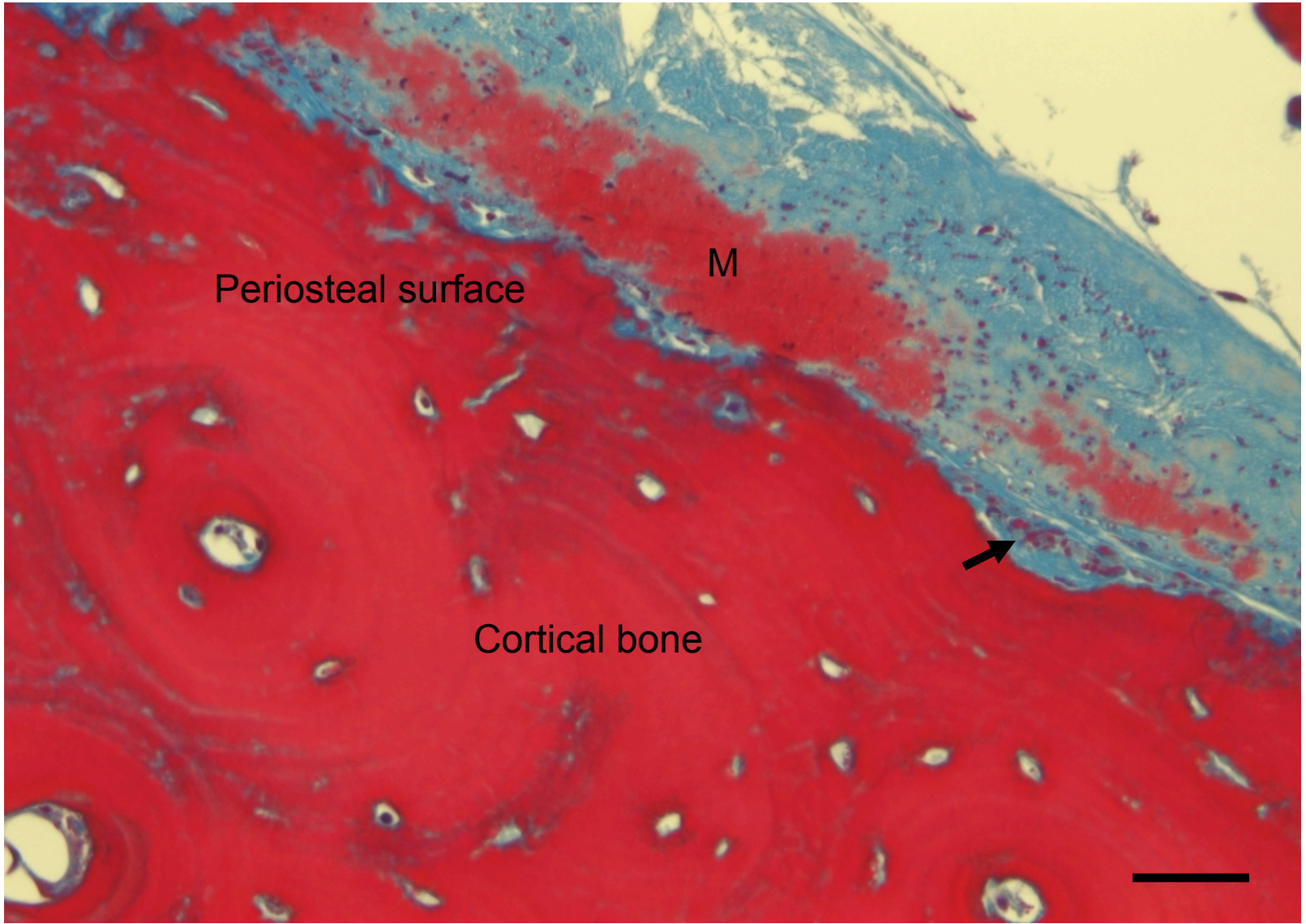


Figure 2

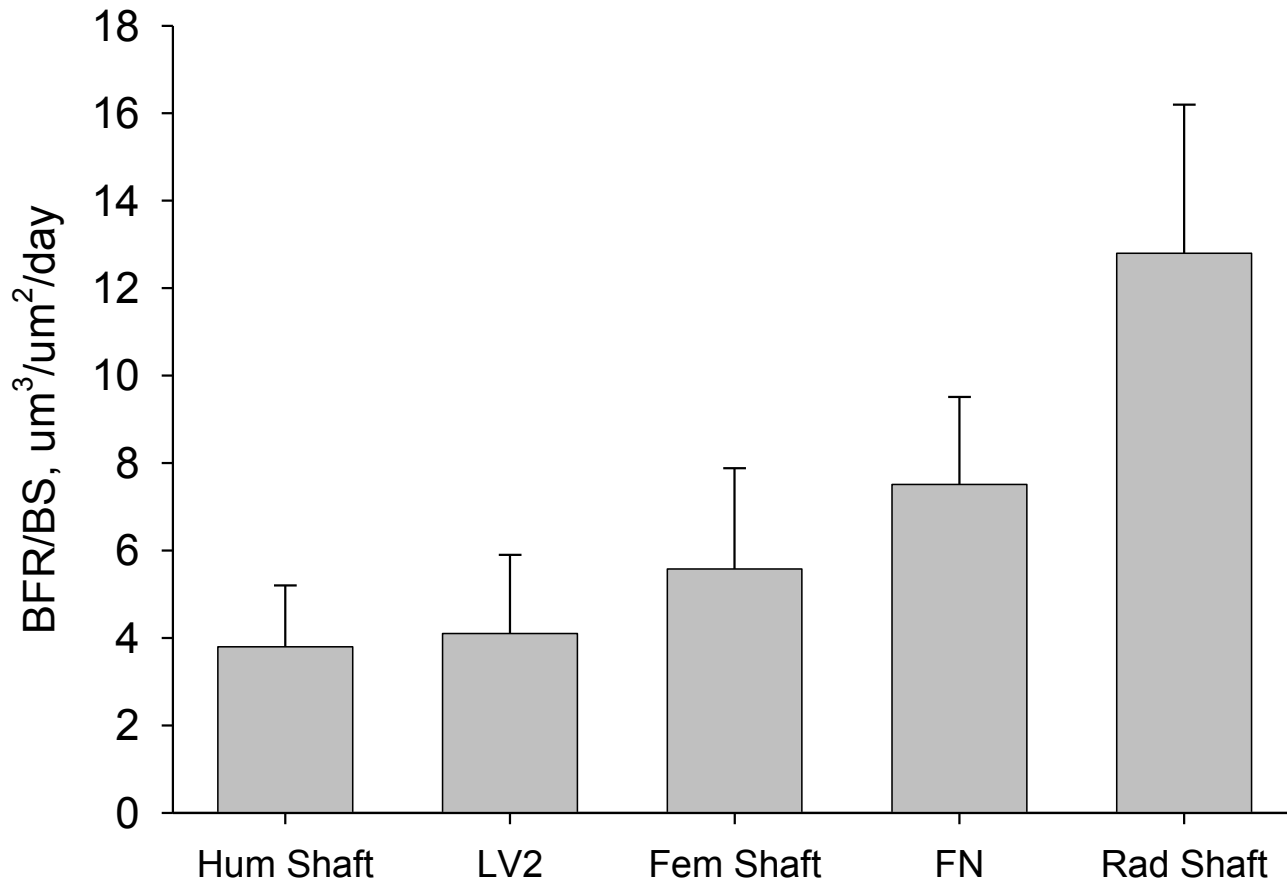


Figure 3