REVIEW

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Co-administration of Antiresorptive and Anabolic Agents: A Missed Opportunity

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ABSTRACT

Co-administration of antiresorptive and anabolic therapies has appeal because these treatments target the two main abnormalities in bone remodeling responsible for bone loss and microstructural deterioration. Antiresorptives reduce the number of basic multicellular units (BMUs) remodeling bone and reduce the volume of bone each BMU resorbs. Intermittent parathyroid hormone (PTH) increases the volume of bone formed by existing BMUs and those generated by PTH administration. PTH also increases bone formation by stimulating the differentiation, maturation, and longevity of osteoblast lineage cells residing upon quiescent bone surfaces. Despite these rationally targeted actions, enthusiasm for this approach waned when combined therapy blunted the increase in areal bone mineral density (aBMD) relative to that produced by PTH. Although many studies have since reported additive effects of combined therapy, whatever the aBMD result (blunting, additive, or null), these outcomes give little, if any, insight into changes in bone's material composition or microstructure and give misleading information concerning the net effects on bone strength. Combined therapy remains a potentially valuable approach to therapy. Because studies of antifracture efficacy comparing combined with single therapy are unlikely to be performed in humans, efforts should be directed toward improving methods of quantifying the net effects of combined therapy on bone's material composition, microarchitecture, and strength. © 2015 American Society for Bone and Mineral Research.

KEY WORDS: ANTIRESORPTIVE THERAPY; ANABOLIC THERAPY; BONE MINERAL DENSITY; MICROARCHITECTURE; OSTEOPOROSIS; REMODELING

Introduction

Antiresorptives reduce bone resorption by reducing the number of basic multicellular units (BMUs) remodeling bone and by decreasing the volume of bone resorbed by each BMU.[1–4] Parathyroid hormone (PTH) acts on existing BMUs and increases the number of new BMUs remodeling bone, facilitating the deposition of more bone than was removed by each remodeling transaction. PTH also stimulates bone formation by promoting the differentiation of flattened osteoblast lineage cells upon quiescent periosteal and endosteal surfaces.[5–7] Thus, combining antiresorptive and anabolic agents should slow microstructural deterioration and at least partly restore bone's microstructure and strength.

When studies comparing combined versus single therapy were designed, it was presumably believed that a greater increase in areal bone mineral density (aBMD) achieved by combined therapy was indicative of greater restoration of bone strength and potentially better antifracture efficacy than single therapy. The corollary must have also been held that there was no advantage in combining therapies if the change in aBMD did not different from, or was less than, the response to either drug alone. These studies were undertaken despite evidence that changes in aBMD in response to most,[8,9] but not all,[10] antiresorptives explain little of their antifracture efficacy.

One of the first reports of blunting of the increase in aBMD by combined therapy was a study of aged ewes given tiludronate plus PTH versus PTH alone.[11] Two subsequent studies suggested that combined therapy blunted the increase in aBMD measured using bone densitometry, trabecular and cortical volumetric BMD (vBMD) measured using quantitative computed tomography, and remodeling markers compared with PTH alone.[12,13] The interpretation of the studies was challenged,[14] but the studies remained influential because they were published in the same issue of the *New England Journal of Medicine* with an editorial that accepted the methodology, the data, and the authors' interpretation.[15]

Several subsequent studies produced different results. PTH plus zoledronate increased aBMD more than PTH alone in the first 6 months and increased BMD less in the second 6 months such that the net aBMD increase did not differ.[16] Denosumab, an inhibitor of receptor activator of NF-κB ligand (RANKL), plus PTH produced additive effects on aBMD and greater benefits in microarchitecture than PTH alone.[17] Nevertheless, combined therapy is not widely regarded as a viable therapeutic option despite there now being many studies reporting additive effects on aBMD.[18–28]

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We suggest that this view is premature. Regardless of whether combined therapy produces an increase, decrease, or no net effect on aBMD, these observations are difficult to interpret. Challenges arise because image acquisition and analysis depend on photon attenuation by the volume of matrix present and its mineral content, features that are independently influenced by antiresorptive and anabolic agents, often in opposite directions. In this Review, we discuss the abnormalities in bone remodeling responsible for microstructural deterioration, the effects of antiresorptive agents alone, intermittent PTH alone, and, finally, the effects of combined therapy.

Reversible and Irreversible Deficits in Bone Matrix Volume and Its Mineral Content

Bone remodeling during young adulthood maintains bone's pristine material composition and microstructure by replacing a volume of old or damaged mineralized bone by an equal volume of osteoid, which then undergoes primary and secondary mineralization.[29] No permanent deficit in matrix volume and its mineral content occurs.[30]

However, two deficits in matrix volume and its mineral content are generated during the remodeling cycle. The first is a reversible remodeling deficit or remodeling 'transient'. It exists because refilling of an excavated cavity is not immediate. The onset of refilling is delayed by about 1 week (the reversal phase),[31] and then matrix is slowly deposited in the cavity, diminishing the deficit in about 3 months. Mineralization of the deposited matrix follows rapidly, within days of matrix deposition, and then proceeds very slowly.[32–35] Fig. 1 shows the constituents and time course of the components of the reversible deficit in matrix volume and its mineral content.

There is always a reversible deficit in matrix and its mineral content; it is ever present but shifting in location because remodeling is continuous with BMUs appearing and disappearing asynchronously as they excavate and refill cavities in different locations, 'turning over' about 10% to 15% of the skeleton annually. The faster the remodeling, the greater the number of BMUs, the greater the number of cavities present at any one time, and so the larger this reversible deficit in matrix volume and its mineral content. The relevance of this deficit is that it plays a pivotal role in determining the accelerated loss of aBMD during early menopause and the accelerated gain in aBMD during early antiresorptive therapy.

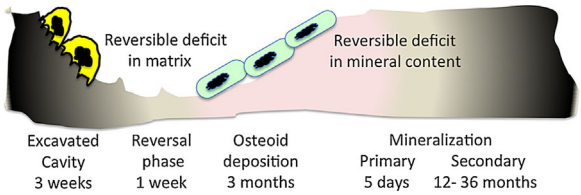

Fig. 1. The basic multicellular unit (BMU). Osteoclasts (yellow) resorb fully mineralized bone (black). After a reversal (resting) phase, osteoblasts (green) deposit unmineralized matrix (osteoid, pink), refilling the cavity slowly. The rapid phase of primary mineralization is followed by a slower phase. The fully reversible deficit in matrix volume and its mineral content produced by the delay and slow formation phase of remodeling is formed by (i) the cavity devoid of matrix or mineral, (ii) the osteoid devoid of mineral, (iii) matrix that has undergone primary mineralization only, and (iv) matrix that has undergone primary but incomplete secondary mineralization (see text).

The second deficit is irreversible. It is a deficit in matrix volume and its mineral content that appears around midlife. It is a consequence of a reduction in the volume of bone formed by the BMU relative to the volume resorbed by that BMU.[36–38] The resulting negative BMU balance is *the* cause of permanent bone loss and permanent cortical and trabecular microstructural deterioration. The negative BMU balance is a target for anabolic agents, not antiresorptives, for reasons to be discussed.

Menopause, Advancing Age, and Microstructural Deterioration

The early rapid decrease in aBMD: the result of increasing the reversible deficit

Before menopause, remodeling is slow and in steady state; the number of cavities at various stages of excavation upon the intracortical, endocortical, and trabecular surfaces approximately equals the number of cavities at various stages of refilling at other locations.[29,30] Remodeling is also balanced with equal volumes of bone resorbed and osteoid deposited by each BMU at a given location (Fig. 2*A*). No matter what the rate of remodeling, there is no permanent bone loss or microstructural deterioration.

At menopause, the number of BMUs excavating cavities upon the intracortical, endocortical, and trabecular surfaces increases, while concurrently, the fewer cavities excavated *before* menopause now enter their refilling phase (Fig. 2*B*). The delay and slowness of refilling of the fewer BMUs initiated before menopause produces this 'perturbation' in steady-state remodeling; more BMUs remove bone from the intracortical, endocortical, and trabecular surfaces than the number of BMUs (generated before menopause) now depositing osteoid at other locations upon these surfaces. This produces the accelerated net decline in aBMD.[32] In a histomorphometric section, the larger surface area participating in bone resorption than the surface area participating in bone formation is a consequence of this differing time sequence of each of the phases of remodeling; this is not 'remodeling imbalance' or 'uncoupling'; these terms refer exclusively to BMU-level resorption and formation.[29,30]

The later slower phase of bone loss: the negative BMU balance and irreversible deficits

If the increased rate of remodeling were the only abnormality produced by menopause, bone loss would cease within 12 to 18 months and aBMD would settle at a new lower level because the many cavities excavated by BMUs in early menopause start refilling and do so completely, while similarly large numbers of BMUs concurrently excavate new cavities at different locations. Steady-state remodeling is restored at a higher remodeling rate with no further decline in aBMD.

However, the increased rate of remodeling is not the only abnormality produced by menopause. Estrogen deficiency is associated with the appearance of a negative BMU balance, which is partly the result of a reduction in the life span of osteoblasts and an increase in the life span of osteoclasts.[39] The many more BMUs now only partly refill the excavated cavities upon intracortical, endocortical, and trabecular surfaces (Fig. 2*C*). Permanent microstructural deterioration results

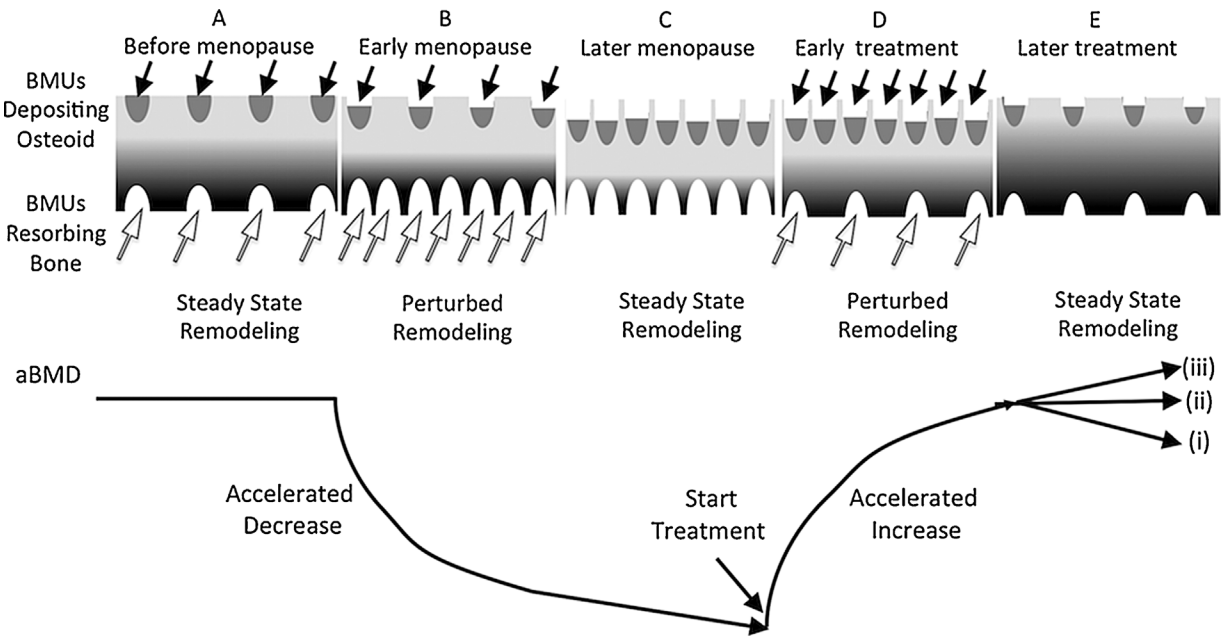

Fig. 2. (A) Before menopause. Remodeling is slow and in steady state. Similar numbers of sites are excavated (white arrows) and completely refilled (black arrows), so no bone loss occurs. **(B) Early menopause.** Remodeling is perturbed. More basic multicellular units (BMUs) resorb bone and each resorbs more bone (white arrows). The fewer cavities excavated before menopause now refill but incompletely (black arrows). Areal bone mineral density (aBMD) decreases rapidly (lower panel). **(C) Later menopause.** Remodeling returns to steady state but at a higher rate. The number of BMUs excavating equals the number (excavated in early menopause) now refilling but incompletely. Bone loss continues but more slowly. **(D) Early antiresorptive therapy.** Remodeling is perturbed. Fewer BMUs excavate smaller cavities (white arrows), while the many more BMUs (excavating in later menopause) refill but incompletely (black arrows). aBMD increases rapidly. **(E) Later antiresorptive therapy.** Remodeling returns to steady state at a slower rate. The fewer and smaller cavities excavated during early antiresorptive treatment refill incompletely as similarly few new BMUs excavate smaller cavities. (i) If the negative BMU balance remains, aBMD slowly declines from its higher level. (ii) If BMU balance is restored, there is no change in aBMD. (iii) If secondary mineralization occurs, aBMD may increase, obscuring continued structural deterioration.

in cortical porosity and thinning, trabecular thinning, and perforation.[40]

Antiresorptive Agents Mainly Target the Reversible Deficit

As their name implies, antiresorptives should slow or stop the decline in aBMD and microstructural deterioration.[1–4] Neither scenarios occur; there is an early rapid increase in aBMD that is often mistakenly interpreted as producing partial reversal of structural deterioration.

The early rapid increase in aBMD and benefits of remodeling suppression

Four events produce the early rapid increase in aBMD. The first two are concurrent and the reciprocal of the events in early menopause; the high number of BMUs resorbing bone decreases, modestly with calcium supplements and selective estrogen receptor modulators (SERMS), more so with bisphosphonates, and most of all with denosumab.[4,40–44] Simultaneously, the many more cavities excavated before treatment now start to refill (Fig. 2*D*). There is now a net increase in matrix volume.

This increase in matrix volume is not an anabolic effect; refilling of the many cavities excavated *before* treatment (which increases matrix volume) is no different from the refilling phase in a placebo arm of a trial. The distinction is that this refilling occurs in the setting of the appearance of fewer new cavities during treatment so fewer cavities offset refilling, producing a net increase in matrix volume by slowing removal of bone. They do *not* build bone; there is no increase in periosteal perimeter enlarging total cross-sectional area, no decrease in endocortical perimeter thickening cortices, and no change in trabecular number, thickness, or connectivity.[44]

The third and fourth events are primary and secondary mineralization. The increase in aBMD is the result of mineralization of the deposited matrix. The rapid phase (primary mineralization) achieves 70% to 80% of complete mineralization within days of matrix deposition.[33–35] The slower phase (secondary mineralization) takes 12 to 36 months to complete. It is the slowest component of the remodeling cycle and the incompleteness of mineralization is part of the ever-present transient remodeling deficit. The mineral content of matrix just deposited and matrix deposited months to years earlier continue to increase.[44] This is relevant to understanding the potentially deleterious effects of long-term antiresorptive treatment[45] and the possible role of PTH co-administration in offsetting this effect by replacing highly mineralized and glycated bone with new osteoid that is later mineralized.[46,47]

Refilling of cavities upon the Haversian canals and endocortical and trabecular surfaces is partial because only the reversible deficit in matrix volume and its mineral content is corrected; the negative BMU balance is either not corrected or only partly

corrected. Nevertheless, partial refilling does reduce porosity, partly restores cortical and trabecular thickness *focally* at the site where the BMU resorbed and the deposited *matrix,* and so reduces stress risers and fracture risk.[48,49] If the negative BMU balance is made positive, producing overfilling of a smaller cavity as suggested with odanacatib,[2] trabecular or cortical thickening above pretreatment values will only be focal.

Restoration of skeletal microstructural deterioration present at the onset of treatment is not feasible because only 10% to 15% of the skeleton is being remodeled annually,[32] even less during antiresorptive treatment. Indeed, the beneficial effect of suppression of remodeling by antiresorptives in slowing microstructural deterioration actually prevents its restoration. If therapy could both overfill cavities *and* increase the rate of remodeling, this would result in focal reconstruction of the skeleton. This is partly the rationale for using PTH therapy.

The later slow increase in aBMD and potentially deleterious effects of remodeling suppression

Ironically, long-term antiresorptive therapy may produce deleterious effects by both suppressing remodeling and by doing so incompletely. By suppressing remodeling, microstructural deterioration is slowed, but material composition may be sacrificed. By incompletely suppressing remodeling, material composition is preserved, but microstructure may be sacrificed.

About 6 to 12 months after starting therapy, remodeling shifts to a new slower steady state. The fewer BMUs (each perhaps excavating a smaller volume of bone) generated during early antiresorptive therapy move into their refilling phase, while concurrently, similarly fewer BMUs excavate new and smaller cavities (Fig. 2*E*). If BMU balance remains negative, matrix and its mineral content are lost, resulting in a slow decline in aBMD from the higher level (Fig. 2, lower panel, line (i)). This decline is reported with weak antiresorptives like calcium supplements and SERMS because remodeling proceeds at 70% to 90% of the pretreatment rate[41–43] (Fig. 3). These drugs have little deleterious effect on material composition.

More efficacious remodeling suppressants like the bisphosphonates and denosumab preserve microstructure because few BMUs excavate new cavities during treatment. Nevertheless, microstructure may be compromised by the slow remodeling with its negative BMU balance and by secondary mineralization; the slowly decreasing matrix volume becomes increasingly fully mineralized (Fig. 2*E*). aBMD may remain stable or increase, thus failing to signal the loss of material and structural strength (Fig. 2, bottom panel, lines (ii) and (iii)).

In addition, increased collagen cross-linking by pentosidine and other advanced glycation end products (AGEs) reduce matrix ductility,[46–48] predisposing to increased microcrack density (owing to reduced removal and increased production). Moreover, if the volume of bone resorbed is reduced by therapy, the osteons formed will be smaller.[50] The relatively larger interstitial bone volume has a higher matrix mineral content, higher AGEs, and accumulates more microdamage than osteonal bone.[51] Several of these deleterious effects may be offset by co-administration of PTH, which may replace older, more mineralized, and more glycated bone with new bone.[47]

The deleterious effects associated with bisphosphonate administration may be largely cortical in origin. For example, zoledronate and ibandronate bind avidly to mineral and may fail to penetrate deeper peri-Haversian cortical matrix.[52–54] The concentrations of these drugs are lower in cortical than trabecular bone. In studies of nonhuman primates, ibandronate reduces endocortical and trabecular surface remodeling, not Haversian canal surface remodeling, and so improves trabecular, not cortical, bone strength[52] (Fig. 4).

Denosumab suppresses remodeling to a greater extent than alendronate and most other bisphosphonates.[55,56] Serum C-terminal telopeptide of type 1 collagen (CTX) is reduced with both drugs; there is almost complete separation of the frequency distribution curves of serum CTX between denosumab-treated women and controls, but about half of the women receiving alendronate had serum CTX no different from controls (Fig. 5*A*). As reported in human subjects and nonhuman primates, upon return of steady state in the second 6 months of therapy, greater intracortical remodeling in the second 6 months of therapy with alendronate than denosumab is likely to account for cessation of the decline in porosity during alendronate therapy (Fig. 5*B*). With time, if remodeling persists during denosumab therapy, lessening of the decline in porosity may occur. Studies of the long-term effects of antiresorptives on porosity are lacking.

Age-related modeling becomes detectable when remodeling is suppressed

Increases in tissue mineral density should become asymptotic after about 3 to 5 years of treatment with full mineralization of matrix.[33–35] Therefore, another mechanism must be responsible for any continued increase in aBMD reported after 5 years' treatment with denosumab and odanacatib (after excluding errors produced by arthritic changes).[57,58]

Bone modeling, the deposition of matrix upon surfaces without prior resorption, may continue in adulthood slowly.[59] Studies of nonhuman primates suggest that age-related bone modeling upon the periosteal and endocortical surfaces is present but remains obscured or is removed by rapid and unbalanced remodeling until that remodeling is suppressed by denosumab or odanacatib.[60,61] If these observations are confirmed in human subjects, there may be greater resistance to bending produced, but cessation or reversal of microstructural deterioration will not be achieved using antiresorptive therapy.

Anabolic Agents Mainly Target the Irreversible Deficit

Reversing microstructural deterioration and bone fragility, 'curing' osteoporosis, requires anabolic therapy. Periosteal apposition increases total cross-sectional area and periosteal perimeter. Deposition of bone upon intracortical surfaces of canals reduces porosity. Endocortical apposition or corticalization of trabeculae reduces medullary cross-sectional area, whereas deposition of bone upon trabecular surfaces thickens them and increases their connectivity.[5,6] There is an absolute increase in mineralized bone matrix volume. This differs from the increase in net mineralized matrix volume produced by fewer cavities offsetting refilling of cavities excavated before starting antiresorptive therapy. Antiresorptives do not modify the external or internal dimensions of bone.

BMU-dependent remodeling-based bone formation

PTH 1-34–and PTH 1-84–mediated osteoid formation and its subsequent mineralization are either remodeling (BMU) based

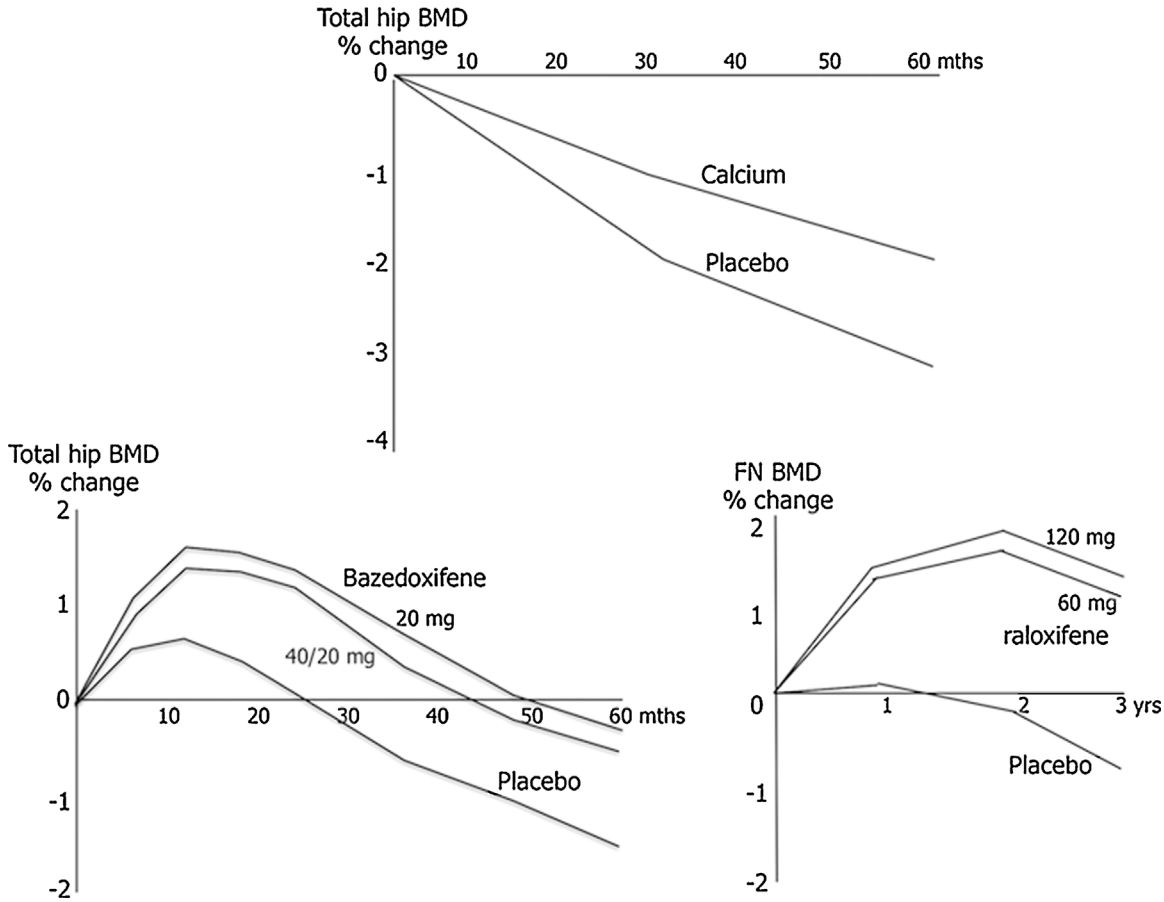

Fig. 3. (Upper graph) With prolonged therapy, total bone mineral density (BMD) decreases using a weak antiresorptive like calcium supplementation because remodeling occurs at about 80% to 90% of the pretreatment rate.[41] (Lower graphs) Total hip and femoral neck (FN) BMD increase during the initial perturbation of remodeling and then decrease in both bazedoxifene (20 mg and 20/40 mg) groups at a rate similar to placebo.[42,43]

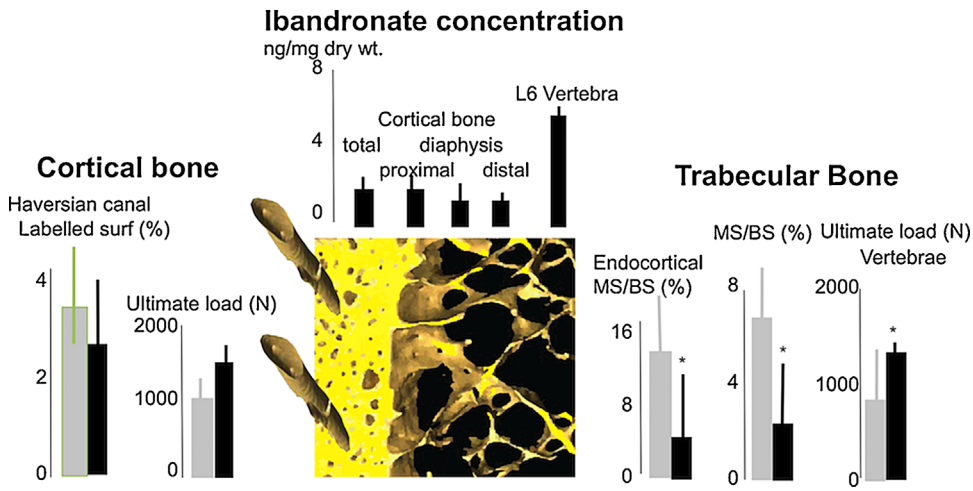

Fig. 4. (Center graph) Ibandronate concentration is lower in cortical than in trabecular bone. (Left graph) Cortical bone. The surface extent of remodeling upon Haversian canals and ultimate load do not differ in treated (black bars) and control (gray bars) groups of nonhuman primates. (Right graph) Trabecular compartment. The surface extent of remodeling upon the endocortical and trabecular surfaces is reduced and the ultimate load tolerated increased in treated, relative to control, animals.[52]

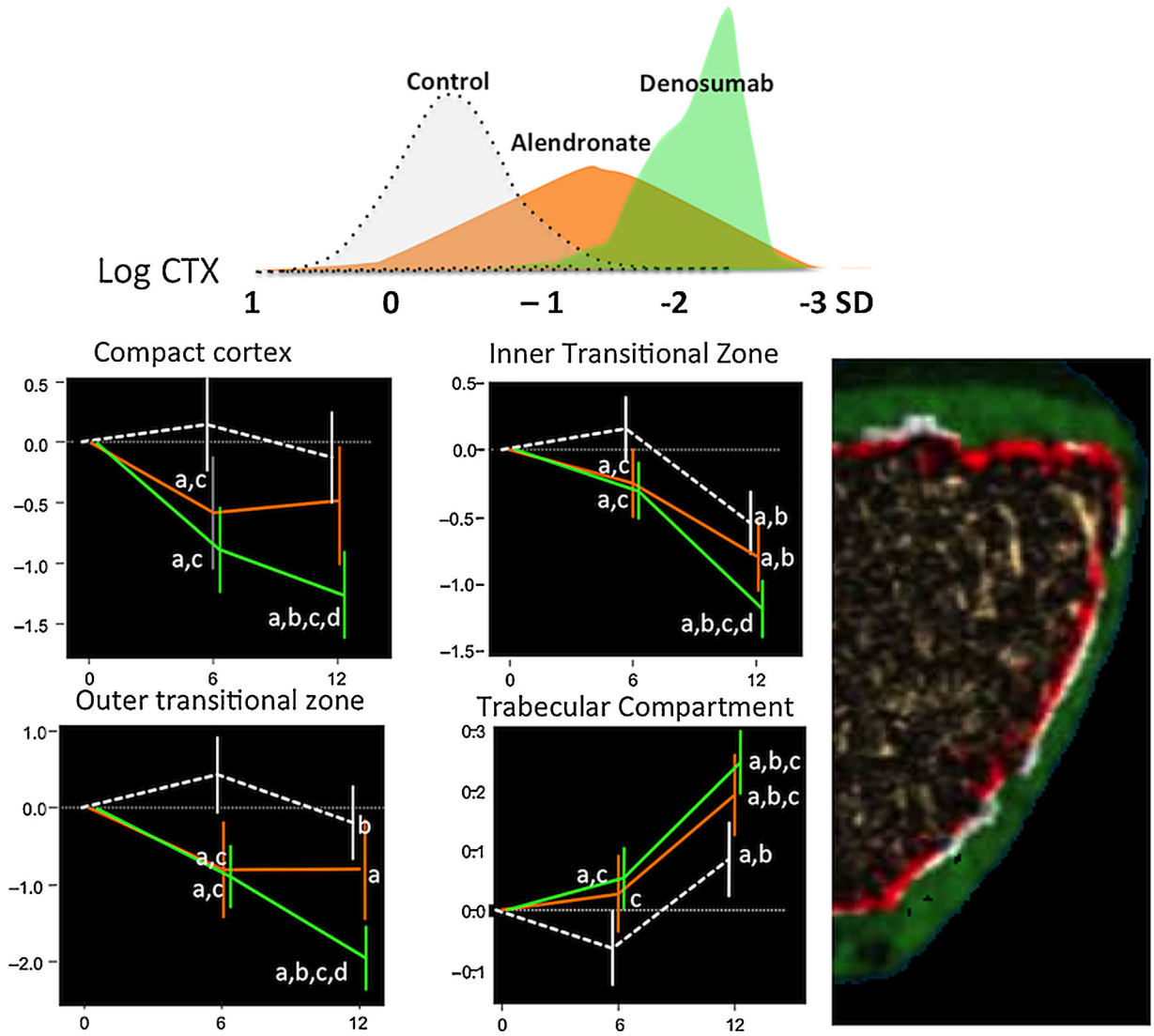

Fig. 5. (Upper panel) Frequency distribution curves for C-terminal cross-linking telopeptides of type 1 collagen (CTX) at 3 months in controls (gray), alendronate-treated (orange), and denosumab-treated (green) women. There is little overlap between denosumab-treated women and controls. There is substantial overlap between alendronate-treated women and controls. (Lower left and center panels) Changes in porosity of the compact, inner, and outer transitional zones and changes in trabecular bone volume fraction at baseline, 6, and 12 months in the three groups. $p < 0.05$ compared with: (a) baseline; (b) month 6; (c) control. (Right panel) Three-dimensional reconstruction of a section of the distal radius with the compact-appearing cortex (green), outer (white), and inner (red) transitional zone and trabecular compartment (yellow).[55]

or modeling based.[62,63,64] About 80% of the total osteoid formed by PTH is remodeling based. The relative contributions from BMUs existing at the time of administration and PTH-mediated newly born BMUs are uncertain, but the more rapid the remodeling, the greater the surface extent of remodeling and the larger the number of BMUs at various stages of remodeling available to PTH.[65]

Whether the BMU is existing at the time of starting PTH or is generated by PTH administration, PTH may achieve its anabolic effect by acting on BMUs in their resorptive, reversal, or formation phases upon intracortical, endocortical, trabecular, and perhaps periosteal surfaces. During the resorptive phase, PTH is likely to promote osteocyte and osteoblast precursor production of RANKL. Subsequent osteoclastogenesis, production of local factors from osteoclasts and matrix they resorb, influence subsequent remodeling events.[14] PTH acting on BMUs in their reversal phase may promote differentiation of osteoblast lineage cells into mature osteoid-producing forms. PTH acting on BMUs in their formation phase is likely to promote bone formation by increasing matrix production and inhibiting apoptosis of osteoblasts.[5,6,65]

Cavities upon endocortical or trabecular surfaces may refill or overfill, in which case the net-positive BMU balance should thicken cortices and trabeculae but still only focally. Anabolic agents like antisclerostin antibody appear to target the large quiescent endosteal and periosteal surfaces.[66,67] PTH inhibition of sclerostin production by osteocytes may contribute to the anabolic action of PTH.[68,69] However, the role in human subjects is not yet established. Genetic ablation of the PTH receptor (PTHR1) in mice increases sclerostin expression and

ablates the sclerostin response to PTH in young sclerostin knockout mice treated before the high bone mass phenotype appears.[69,70] The anabolic action of PTH is lessened in transgenic mice overexpressing human sclerostin, and these mice develop osteopenia with age.[71] When these mice are treated with intermittent PTH, trabecular bone volume did not increase, even though the same dose increased trabecular bone volume and suppressed endogenous sclerostin mRNA in wild-type mice. Thus, overexpression of SOST impairs the full anabolic action of PTH. Therefore, mouse genetic and pharmacologic data are highly suggestive. Data in human subjects concerning the role of PTH-mediated sclerostin inhibition for its anabolic action is awaited.

BMU-independent modeling-based bone formation upon the periosteal and endosteal surfaces

Periosteal surface

Periosteal apposition is vigorous during growth in human subjects and in animal models, but it is modest during adulthood and difficult to detect.[59] There is no evidence that intermittent PTH produces measurable changes in periosteal circumference, even though there is evidence in some studies of increased apposition using quadruple labeling procedures.[62,63] Deposition of bone at this location is advantageous because of the disproportionate increase in bending strength achieved by a small increment in bone diameter;[48] however, robust evidence for this is lacking.

Endocortical surface

About 80% of the three (intracortical, endocortical, trabecular) components of the endosteal surface is quiescent and provides a vast surface area upon which osteoid can be deposited. In the mouse, osteoblast lining cells differentiate into osteoblast matrix-forming cells that also mineralize the newly deposited osteoid.[7]

PTH increases cortical area.[5,6] If an anabolic effect on periosteal apposition in adult human subjects is minimal, then the increase in cortical area and thickness could be explained by bone formation upon the endocortical surface. Although this is held to be the case, it is not consistently observed. Although evidence is lacking, it is plausible that bone formation upon the surfaces of adjacent trabeculae abutting the endocortical surface cause them to coalesce, a process referred to as trabecular corticalization described during growth[72] and the opposite of cortical trabecularization.[40]

Intracortical surface

Modeling-based bone formation upon intracortical canal surfaces may reduce canal diameter focally, increasing the proportion of the osteonal area that is matrix while reciprocally decreasing the area that is void volume. However, many studies suggest the opposite; cortical porosity increases during early intermittent PTH therapy.[73–75]

Porosity may increase as newly initiated BMUs excavate fully mineralized bone at points upon canal surfaces. Porosity may be transient because in subsequent deposition of osteoid, its primary, then slower secondary mineralization should refill or overfill the cavity excavated upon the canal surface, reducing porosity, but evidence for this is lacking. Alternatively, the increase in porosity may be partly factitious. Bone formation upon trabeculae abutting against the cortex may cause them to thicken and coalesce, the opposite of trabecularization of cortices. Incomplete coalescence may produce a 'pseudo porosity' as trabeculae remain separated by what appears to be porosity within the inner transitional zone of the cortex adjacent to the medullary canal. Work is needed to examine this mechanism.

Trabecular surface

An increase in trabecular thickening with increased connectivity is probably the best-documented morphological effect of intermittent PTH therapy.[5,6] This may be undetected by noninvasive imaging methods that depend on photon attenuation because newly synthesized matrix may transmit, rather than attenuate, photons as discussed recently.[76]

The myth of the anabolic window

The earlier rise in N-terminal propeptide of type 1 collagen (P1NP) and later rise in circulating CTX have led to the widely stated view that there is an "anabolic window," a period of time when the actions of PTH are maximally anabolic.[77]

Before considering the veracity of this notion, several issues need to be addressed concerning the use of remodeling markers to define any anabolic window. There is virtually universal use of the terms 'resorption' and 'formation' markers when referring to circulating measures of bone remodeling. This implies that measurements like serum CTX and P1NP are accurate surrogates of the volumes of bone resorbed from bone surfaces or added upon them, respectively. This has never been demonstrated. This terminology appears to be based on robust evidence that cleavage of these peptides occurs in association with resorption and formation, respectively,[78] but finding correlations of $\sim$0.4 between the markers and the extent of bone formation and resorption (using histomorphometry) and bone turnover (using isotopic methods) is based on small studies with wide scatter of the values such that little of the variance in these gold standards are explained by a circulating marker level.[78,79]

Circulating levels of CTX and P1NP are a function of the number of BMUs remodeling the total cortical and trabecular bone volume, the volumes of bone resorbed and deposited by each BMU, and the clearance of these peptides. A decrease in remodeling rate produced by antiresorptive therapy is a more important determinant of the circulating concentration of a marker than the difference in the volumes of matrix resorbed and deposited by each of the fewer BMUs now remodeling bone. However, it is the difference in the volumes of matrix formed and resorbed by each BMU that determines whether there is net deposition of osteoid upon, or removal of bone from, a surface. If remodeling is rapid, the surface extents of resorption and formation in a bone biopsy will be large, and circulating markers will be high, but neither inform as to whether bone is lost or gained. Attempts at using ratios of the markers as surrogates of BMU balance are also flawed for these reasons, especially because of the differing time course of resorption and formation phases of remodeling.[80,81]

When PTH is administered, there is a rapid rise in P1NP, reflecting stimulation of osteoid formation by BMUs in their formation phase, direct modeling-based bone formation, or both. There is a more gradual rise in CTX, which is said to be shifted to the right, ie, occurs later. The so-called gap between

the two is referred to as the anabolic window. However, it is difficult to identify data on which this claim might be based. Our examination of published data in a large number of studies failed to find any consistent 'gap' that could be interpreted as such a 'window'.[17–23] Frequency distribution curves for the remodeling markers overlap to a major extent. Although CTX and other "resorption" markers are often shifted to the right, there is no evidence of a bimodal distribution.

The notion of the anabolic window may be based on the view that PTH initially promotes modeling-based bone formation and results in resorption by stimulating BMU-based remodeling. If so, such a switch seems an unlikely biological event. A more parsimonious interpretation is that PTH has a direct action on osteoblast lineage cells in existing BMUs or newly initiated BMUs, resulting in rapid release of cell-derived P1NP. With each activated BMU, resorptive removal of mineralized matrix increases CTX, which eventually reaches levels that can be measured in the circulation.

A concern with the concept of an anabolic window is that it may create a perception that PTH will no longer deposit osteoid or will deposit less osteoid after 'resorption' markers increase. There is a lessening of the effect of PTH, but the reasons for this are unknown. There is no evidence that the lessening is signaled by a rise in 'resorption' markers or caused by bone resorption. Increased remodeling rate increases both CTX and P1NP. If each of the many new BMUs produces a positive balance, deposition of newly synthesized osteoid will occur even though serum CTX is high.

Combined Therapy

Combined therapy and aBMD

PTH exerts its anabolic effect predominantly by increasing the number and activity of BMUs.[62–64] Because bone resorption is an essential part of the activity of a BMU, what is there about the action of PTH that equips it to promote bone formation when combined with antiresorptive treatment, which reduces the number of BMUs and, in the case of RANKL inhibitors like denosumab or osteoprotegerin, virtually obliterates them?

PTH acts directly on osteoblast lineage cells to increase differentiation of committed precursors that have reached the stage of expressing functional PTHR1, as well as inhibiting apoptosis of osteoblasts and osteocytes.[65,82] PTH also could exert these functions at sites that have not undergone prior resorption (a modeling effect); lining cells are a target because these cells are capable of being awakened to resume anabolic activity.[7] Furthermore, PTH rapidly reduces production of sclerostin by osteocytes, an effect shown in the mouse (but not yet in human subjects) to be required for full expression of anabolic activity. This is also free of any requirement for osteoclasts and could provide another pathway to a PTH-mediated anabolic effect in the presence of inhibited resorption.[69,70]

Thus, there is a rationale for combined therapy. However, quantifying the effects of combined therapy using noninvasive imaging is challenging. The mineralized matrix volume of a region depends on the matrix volume and its mineral content. Bone densitometry cannot distinguish whether a change in aBMD is the result of a change in matrix volume, its mineral content, or both. Antiresorptive and anabolic agents produce changes in these traits that differ in magnitude and direction so that the net bone strength resulting cannot be predicted from the change in aBMD.

For example, intermittent PTH may increase matrix volume but reduce its net mineral content by replacing fully mineralized bone with a larger volume of osteoid or by depositing osteoid upon a surface. Antiresorptives reduce remodeling rate, so the less remodeled matrix undergoes more complete secondary mineralization. However, any residual remodeling produces bone loss (if BMU balance is negative), so the ever-decreasing matrix volume has an increasing mineral content.

If combined therapy produced a net increase matrix volume but a net decrease in its mineral content resulting in a decrease in aBMD, this is likely to be interpreted as bone 'loss', even though matrix volume increased. If combined therapy produces no net change or a decrease in matrix volume but a net increase in its mineral content resulting in an increase in aBMD, this is likely to be interpreted as deposition of bone matrix and increased strength when more complete secondary mineralization may make bone more brittle.

Co-administration of alendronate and PTH or tiludronate and PTH are reported to blunt the increase in aBMD relative to PTH alone.[11–13] Co-administration of zoledronate and PTH is reported to be initially additive and then to produce less increase in aBMD, so no net difference in spine aBMD was observed at 12 months.[16] If blunting of the aBMD response to PTH was due to there being fewer BMUs for PTH to act upon during antiresorptive therapy, then blunting should be *more* severe with co-administration of PTH with zoledronate, denosumab, or osteoprotegerin (OPG) than with alendronate. The opposite is reported,[17] and many studies report additive effects.[17–27]

An alternative explanation for the differing effects of various combined regimens on aBMD may be that the responses depend mainly on differences in the antiresorptives themselves, rather than differences in numbers of BMUs they leave unsuppressed to provide osteoblast lineage cells for PTH to act upon. For example, denosumab reduces remodeling and increases aBMD more than alendronate because fewer BMUs are left to remodel bone than with alendronate.[40] If blunting is because of fewer BMUs for PTH to act upon, then blunting should be *greater* with denosumab/PTH than alendronate/PTH (and both should blunt changes in morphology relative to PTH alone).

This is not observed. Additive effects on aBMD are reported with PTH/denosumab versus PTH.[17,83] Additive effects have also been reported comparing PTH/OPG versus PTH.[23,28,84] Studies directly comparing PTH/alendronate versus PTH/denosumab are not available, but comparisons with PTH/OPG are published.[23,28] Although suppression of remodeling markers and the surface extent of remodeling (measured using histomorphometry) are greater with OPG alone or PTH/OPG than alendronate alone or PTH/alendronate, there was no evidence of blunting of morphology or bone strength.[23] On the contrary, both PTH/alendronate and PTH/OPG more greatly *increased* trabecular bone volume, resistance to bending, maximum force needed to fracture the femur, and work to failure than alendronate, OPG, or PTH alone, and the effects on morphology and strength of the combined regimens did not differ from each other.[23]

Combined therapy and bone microarchitecture

The difficulties in evaluating the effects of combined therapy using bone densitometry and aBMD might be partly overcome by independently measuring changes in matrix volume, mineral density, and the microarchitecture assembled by this mineralized matrix volume. However, like bone densitometry, image

acquisition and analysis using HRpQCT also depends on photon attenuation by a region's matrix volume and its mineral content.

Herein lies the challenge. Quantification of microstructure depends on the mineral content of the matrix used to assemble it. Examples of the difficulties that arise as a consequence of this was the subject of a recent publication,[77] but issues arising in the setting of combined therapy were not addressed and are illustrated from the well-designed and executed study by Tsai and colleagues.[17]

Tsai and colleagues[17] report that combined therapy increased cortical vBMD, increased cortical matrix mineral density, had no effect on porosity, and increased cortical thickness (Fig. 6). These net changes in morphology are not explained by the changes reported with each treatment alone. PTH reduced cortical vBMD, perhaps because of the reported decrease in matrix mineral density and the increase in cortical porosity. If accepted on face value, this leaves the increase in cortical vBMD attributable to the increase produced by denosumab. However, denosumab did not increase cortical matrix mineral density or decrease cortical porosity, leaving the increase in cortical vBMD by denosumab unexplained. Combined therapy increased cortical matrix mineral density, but PTH decreased it and denosumab had no effect. Cortical porosity was unchanged with combined therapy but increased with PTH and was unchanged with denosumab. Cortical thickness increased with combined therapy and with denosumab, an antiresorptive agent, but was unchanged with PTH.

It is likely that in the PTH-treated subjects, the reduced cortical vBMD, reduced matrix mineral density, increased porosity, and failure to detect increased cortical thickness are the result of replacement of mineralized bone with younger bone or addition of osteoid not detected by threshold-based image analysis.[77] Most pores are $<$100 microns.[85] At a resolution of 130 microns, voxels containing a pore or part of a pore also contain matrix and so attenuate photons above the threshold designated as 'porosity'. Increased secondary mineralization facilitated by denosumab could have altered edge detection, erroneously suggesting cortical thickness increased.

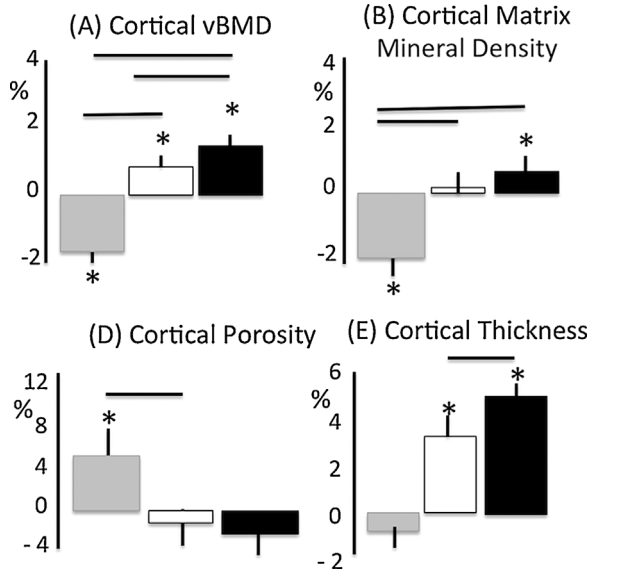

Fig. 6. Tibial cortical volumetric bone mineral density (vBMD; *A*), matrix mineral density (*B*), porosity (*C*), and thickness (*D*). Percent changes relative to baseline after 12 months of intermittent teriparatide (TPTD, gray bars), denosumab (white bars), or both (black bars). *$p < 0.05$ relative to baseline. Horizontal lines denote significance between group differences. Adapted from parts of Figs. 2 and 3.

Summary and Conclusions

There are several cogent reasons for considering combined therapy as a viable addition to current approaches to treatment. First, a negative BMU balance is the cause of structural deterioration and is due to an imbalance in the volumes of bone resorbed and deposited by each BMU. Antiresorptives reduce the volume of bone resorbed by each BMU, PTH may increase the volume of bone resorbed, and so coadministration is likely to lessen the negative BMU balance or make it positive. Second, after menopause, large numbers of BMUs initiated at points upon the three (intracortical, endocortical and trabecular) components of the endosteal surface, each with their negative BMU balance, erode bone's microstructure. Antiresorptives reduce the number of BMUs remodeling bone. PTH increases them but produces a positive BMU balance resulting in net deposition of bone matrix. Third, ageing is associated with reduced periosteal bone formation. Antiresorptives do not appear modify cellular activity upon the periosteal surface but PTH may increase modeling based bone formation upon this surface and upon the endocortical surface.

Fourth, antiresorptives may have deleterious effects. They slow but do not stop remodeling. The component of remodeling that continues, particularly in cortical bone, is likely to compromise microstructure. The component of remodeling that is suppressed may compromise bone's material composition. These limitations probably contribute to the modest 50–60% reduction in vertebral and hip fracture risk, and meager 20–30% reduction in non-vertebral fractures. Adding PTH may partly restore bone mass and microarchitecture and replace more densely mineralized and glycated collagen with new bone matrix. While randomized double blind trials with fracture outcomes are lacking, studies in animals and human subjects report additive effects of combined therapy when outcomes are based on measurement methods that are free of challenges in noninvasive image acquisition and analysis,[76] such as dynamic histomorphometry and mechanical testing. Combined antiresorptive and anabolic therapy should not be discounted as an option for patients at risk for fracture

Disclosures

ES is one of the inventors of Strax 1.0, an algorithm used to quantify bone microstructure, and is a director of Straxcorp. TJM states that he has no conflicts of interest.

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