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MEETING REPORTS

Meeting Report from the 27th Annual Meeting of the American Society for Bone and Mineral Research

September 23-27, 2005 in Nashville, Tennessee, USA

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- On The Road to the "Big" Chondrocyte – Ernestina Schipani
- Old and New Molecules in Osteoclast Biology – Alberta Zallone
- Wnt and Friends – Gordon J. Strewler
- Phosphate, Klotho, FGF23 and More – Gordon J. Strewler
- Vitamin D Research – J. Wesley Pike
- Genetics of Osteoporosis – Serge Ferrari
- Imaging Assessment of Bone – Yebin Jiang
- Treatment of Osteoporosis – Ego Seeman

ON THE ROAD TO THE "BIG" CHONDROCYTE

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Chondrocyte hypertrophy is a key event in endochondral bone development. Chondrocytes first become "big" or "hypertrophic" and then die and get replaced by bone. At the 2005 ASBMR meeting, numerous papers discussed signaling pathways involved in chondrocyte hypertrophy. In particular, both *in vivo* and *in vitro* approaches have proven convincingly that Wnts and β-catenin play a significant role in this process (1-4). A number of exciting studies published earlier in the year had already demonstrated that the canonical Wnt pathway is required for proper commitment of osteochondroprogenitor cells towards the osteoblast or the chondrocyte lineage (5-8). Papers on Wnts and chondrocytes presented at the meeting showed that the Wnt canonical pathway is also critically important for terminal chondrocyte differentiation. In particular, mice lacking β-catenin in chondrocytes have a severe delay of chondrocyte hypertrophy (1); conversely, overexpression of β-catenin *in vitro* induces chondrocyte hypertrophy through mechanisms that may involve regulation of Runx2 expression (2). Consistent with these data, *in vivo* overexpression in chondrocytes of the inhibitor of β-catenin/TCF4 (ICAT) leads to delayed endochondral bone development and chondrocyte maturation (4). Intriguingly, however, ablation of sFRP1, an antagonist of Wnt signaling in mice, is associated with delay rather then acceleration of chondrocyte terminal differentiation, at least *in vitro* (3). The Wnt world is obviously complex, and its relationship to chondrocyte hypertrophy needs to be fully elucidated.

It is well known that a lack of PTHrP or Ihh leads to severe and premature hypertrophy (9), leading to an obvious question for bone: is there any relation between PTHrP/Ihh and Wnts in chondrocytes? Preliminary data suggest that a relationship between the two pathways does exist and that it is an antagonistic one (1).

Whereas β-catenin is required for hypertrophy, the well-known trio of transcription factors SOX9, SOX5 and SOX6 inhibits the process. At this ASBMR meeting, elegant data were presented that demonstrate that, at least *in vitro*, this inhibition involves the SOX-dependent, up-

regulation of members of the S100 family of kinases, S100A1 and S100B (10). The fog surrounding downstream effectors of SOX activity in cartilage is getting less thick, but we are still missing the upstream regulators of the SOX trio. Interesting and intriguing new data provide evidence that *in vivo* up-regulation of p38 signaling regulates SOX9 activity (11). Similar to the effects of SOX9, *in vivo* constitutive activation of p38 signaling in chondrocytes delays terminal chondrocyte differentiation (11) and formation of both the primary and the secondary ossification center. However, in contrast to what has been reported for other classical inhibitors of hypertrophy, such as Ihh, increased p38 activity is associated with inhibition rather than stimulation of proliferation. This finding is quite interesting, especially in light of previous data demonstrating that increased ERK1/2 activity inhibits hypertrophy with no effect on proliferation (12). Taken together, these data suggest that p38 and ERK1/2 have important but distinct roles in chondrogenesis.

Notably, hypoxia also promotes early chondrogenesis and suppresses chondrocyte terminal differentiation via a complex molecular mechanism involving HDC4 and p38 (13), and independently of the SOX trio. These novel and interesting data add to the lines of evidence indicating that hypoxia is involved not only in the proliferation and death of chondrocytes, but also in their differentiation and hypertrophy, as was previously suggested (14).

In additional studies relevant to hypertrophy, new data about an old player, retinoic acid (RA), show that, at least *in vitro*, RA positively modulates collagen type X (15). New data about a recent player, the transcription factor Dlx2, indicate that in both *in vitro* and *in vivo* assays, Dlx2 is a downstream target of BMP2 action and induces expression of collagen type X (16).

Studies of human diseases reported at this meeting revealed interesting lessons about chondrocyte hypertrophy. In mice, the knock-in of collagen type X mutations previously identified as the cause of Schmid metaphyseal chondrodysplasia (17) generates a phenotype that closely mimics the human disease and is similar to the transgenic phenotype of mice overexpressing similar mutations (18). The transcriptional repressor TRPS1, associated with the tricho-rhino-phalangeal syndrome (TRPS), was implicated in chondrocyte terminal differentiation by experiments in which mice null for TRPS1 show delayed transition from cartilage to bone via effects on Runx2 expression and activity (19).

A critical step in endochondral bone development is the replacement of cartilage by bone. Conditional knockout of VDR in chondrocytes revealed that VDR is critical in this transition; VDR upregulates RANKL in hypertrophic chondrocytes, and thus controls osteoclast activity at the border between cartilage and bone (20). This finding sheds new light onto the pathogenesis of rickets. Talking about cartilage-to-bone transition brings VEGF to mind. Interestingly, transgenic overexpression of VEGF in the growth plate seems not to interfere with cartilage development *per se*, but rather with bone collar formation and cortical bone development (21). In particular, the mutant mice show an aberrant formation of excess cortical bone with exuberant intracortical remodeling, ending in a trabecularization of the cortical bone itself and extreme deformities. Is this dramatic phenotype the result of an ectopic expression of VEGF in cells of the osteoblast lineage, or rather the result of an interesting cross-talk between the hypertrophic chondrocyte and cells of the bone collar? Regardless, a warning is there: too much VEGF is not good for bone. It is accepted that hypertrophic chondrocytes undergo apoptosis. However, ectopic cell death in the proliferative layer of the growing growth plate can also occur in the absence of hypertrophy. This is apparently what is going on in mice that lack the protein kinase Nek1 and are dwarf (22).

Critical players in cartilage development during fetal life are not necessarily critical in postnatal growth. However, this is the case for Ihh. Study of a clever conditional knockout of Ihh exclusively in postnatal cartilage demonstrated that Ihh is also a critical gatekeeper of the hypertrophic zone

postnatally (23). It will be interesting to investigate whether in postnatal life, as during development, the inhibitory effect of Ihh on hypertrophy is mediated through up-regulation of PTHrP. With regard to PTHrP and Ihh, it is still an open question whether prenatally Ihh directly up-regulates PTHrP expression in round proliferative chondrocytes adjacent to the articular surface, as suggested by genetic manipulations (24), or rather acts via an intermediate such as BMP2, as suggested by *in vitro* studies of promoter activity (25).

If hypertrophy takes place at the wrong place, a pathological state can be generated. Ectopic and disorganized hypertrophy can transform a healthy articular surface into an “osteoarthritic” one. This is what happens when Smurf2, an E3 ubiquitin ligase, is overexpressed in cartilage (26). Hypertrophy is not good news for the nucleus pulposus either. When Runx1 or Runx2 is overexpressed in cartilage, cells in the intervertebral disk undergo hypertrophic differentiation with disk degeneration, severe kyphosis and scoliosis (27).

Our knowledge of factors and signaling pathways that regulate hypertrophy is clearly expanding and deepening. The challenge now is to draw a unifying working hypothesis, if possible, and to identify the cellular mechanisms that ultimately lead to hypertrophy. In other words, what is the ultimate effector(s) that all these signaling pathways are controlling that transforms a chondrocyte into a “big”, “hypertrophic” one?

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OLD AND NEW MOLECULES IN OSTEOCLAST BIOLOGY

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Recently, bone-specific roles have been identified for molecules already known for their effects on other organs. The most recent such molecule is follicle stimulating hormone (FSH), known as a regulator of estrogen levels, that is reported to directly enhance osteoclast formation and survival (28;29). FSH and FSH receptor (FSHR) null mice, despite severe hypogonadism, have normal bone mass not different from their wild-type littermates. FSHR is expressed by osteoclasts. In the presence of physiological levels of FSH, RANKL-induced osteoclast formation was enhanced and, if FSHR was over-expressed, the effects were strongly potentiated. AKT, a key anti-apoptotic kinase, was immediately phosphorylated, as were the MAP kinases Erk1 and Erk2. This was paralleled by an increased nuclear localization of c-Fos at 30 minutes. Both effects were pertussis tussin inhibitable. In the pit assay, FSH directly stimulated bone resorption in a dose-dependent manner. This effect was paralleled by enhancement of actin ring formation and acid secretion. These results suggest that while estrogen directly controls bone cell apoptosis, the loss of bone observed in hypogonadism conditions could be due more to the rise of FSH than to the low levels of estrogens.

The direct effect of estrogen on the birth and apoptosis of bone cells was confirmed in mice in which ERα cannot interact with DNA (30). Previous results indicated that the classical genotropic actions of sex steroids are dispensable for their bone protective effects. The data presented confirmed very elegantly the non-genotropic actions of estrogens on bone cells: as expected, at three months these mice had an atrophic uterus and ovariectomy had no effect. However the injection of estrogen was followed by ERK phosphorylation and ERK-regulated transcription of ELK-1 in vertebral lysates, indicating the effectiveness of nongenomic signals. After estrogen treatment, osteoclastogenesis was also augmented in *ex-vivo* cultures, both in wild-type and transgenic mice, while osteoclast apoptosis was increased.

A new role for oxytocin was also presented (31). This hormone is produced locally, in an estrogen-dependent way, by both kinds of bone cells and has an autocrine-paracrine action. Its combined effects keep the level of bone turnover high. The fact that the oxytocin KO mice present thicker bones compared to wild-type littermates suggests

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that perhaps the maintenance of the physiological level of osteoclastogenesis is the prevailing effect of oxytocin.

Calcitonin is a well-known inhibitor of osteoclast activity, but many findings in patients after thyroidectomy have remained unexplained. The study of Calc-1 deficient mice who express neither calcitonin nor α-CGRP is relevant to this point (32). A high bone mass was observed at ages ranging from 3 to 18 months, whereas α-CGRP-deficient mice were osteopenic. The results indicate a previously undetected role of calcitonin as a regulator of bone turnover that affects both bone resorption and formation. How the effects on bone formation are exerted is unknown. A puzzling, related result is that knock-down of calcitonin receptor in mice results in decreased trabecular bone volume only in young females, and is associated with increased bone resorption (33).

Stromal derived factor (SDF-1) has emerged as a key molecule involved in the recruitment of putative pre-osteoclasts from the blood (34), although what occurs in inflammation is still a matter of debate. TNF, which potently stimulates osteoclast formation, seems to inhibit SDF-1 secretion (35).

Osteoclasts appear more and more to be not only the effectors of bone resorption, but also part of a finely tuned mechanism capable of controlling the amount of bone that will be resorbed. A negative feedback is induced in osteoclasts by RANKL (36) through the induction of iNOS/NO. The signal mechanism responsible for NO secretion appears to involve IFN-β and P-Stat-1. Antibodies against IFN-β were able to abolish the effect. *Stat1*(-/-) mice and mice deficient in the interferon-β receptor both exhibited little NO response to RANKL.

Conflict of Interest: The author reports that no conflict of interest exists.

WNT AND FRIENDS

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Center stage at the 2005 Annual Meeting of ASBMR was held by Wnt, which emerges as an actor in early mesenchymal cell fate decisions, cartilage, bone remodeling, and cancer, but PTHrP also made a brief appearance.

The role of canonical Wnt signaling in early development was tested by removing β-catenin, a key mediator in the canonical pathway, from mesenchymal condensations with *dermo1-cre* (37). This resulted in ectopic chondrocyte formation at the expense of osteoblasts, suggesting that canonical Wnt signaling plays a role in early cell fate decisions, consistent with earlier results (38). Wnt3A inhibited chondrogenesis in micromass cultures but promoted chondrocyte hypertrophy, and exposure to the Wnt inhibitor Dickkopf-1 (Dkk1) had opposite effects (39). Viral overexpression of Wnt family members in embryonic chick sternal cartilage led to the conclusion that canonical Wnt signaling enhances chondrocyte hypertrophy, but Wnt signaling through the calcium pathway represses chondrocyte maturation (2).

Deletion of the *dkk1* gene is lethal, but heterozygotes have a high bone mass phenotype without other deleterious features (40) – a result that might have been anticipated, considering that the HBM mutation affecting LRP5 seems to impair inhibition of Wnt signaling by Dkk proteins. Overexpression of Dkk1 or 2 in osteoblasts inhibits mineralized nodule formation and promotes adipogenesis. These results contrast with the recently reported knockout of Dkk2, which produces a low bone mass phenotype that apparently reflects a positive effect of Dkk2 on late stages of osteoblast differentiation (41).

The therapeutic potential of the Wnt pathway in osteoporosis was also addressed. Mapping of the Dkk-binding domain on LRP5 permitted *in silico* screening for small molecules that would disrupt this interaction (42), with the identification of multiple compounds that reversed Dkk-mediated inhibition of Wnt

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signaling at low micromolar concentration and simulated bone formation when injected onto mouse calvaria. PTH treatment reduces Dkk expression, raising the possibility that Dkk inhibition mediates anabolic effects of PTH, but PTH signaling through the Wnt pathway was maintained in mice that overexpressed Dkk1 in osteoblasts, as well as in MC3T3 cells stably transfected with Dkk1 (43). Moreover, treatment with an anabolic PTH regimen increased BMD in mice that overexpressed Dkk1 in osteoblasts (44).

Wnt signals are induced by expression of the transcription factor Msx2. Wnt expression is increased in cells transduced with Msx2 and the canonical Wnt pathway is activated in surrounding cells (45). In CMV-Msx2 transgenic mice, bone density is increased and body fat diminished, and osteoblasts from these mice display markedly increased signaling through the canonical Wnt pathway.

Multiple myeloma cells express high levels of Dkk1, and it was previously proposed that Dkk1 expression underlies the well-known inhibition of bone formation in osteolytic lesions of myeloma (46). In SCID-rab mice with primary human myeloma cells implanted in bone, treatment with a neutralizing Dkk1 antibody markedly increased bone mass and osteoblast number (47). In addition, osteoclast number and tumor burden were both diminished, suggesting that Dkk1 has a central role in both the osteolytic phase of multiple myeloma and the uncoupling of bone destruction from the usual osteoblastic response. In bone explants, Wnt3A inhibited osteoclastic bone resorption, and Dkk1 reversed this inhibition, while also blocking the stimulatory effect of Wnt3A on new bone formation (48), again consistent with the notion that Dkk mediates both osteolysis and impaired bone formation in multiple myeloma.

The potential role of PTHrP deficiency in osteoporosis has hitherto received little attention, but nuclear signals from PTHrP may be important in the aging of bone and other tissues as well. To assess the physiological role of the midregion and carboxyl-terminal domain of PTHrP, which contains a nuclear localization signal, PTHrP(1-84) was “knocked in” in place of wt PTHrP(1-141) (49). These mice underwent premature senescence, beginning *in utero*, with osteoporosis, skin thinning, unstable gait and cachexia postnatally. Senescence marker genes were prematurely expressed in many tissues, even *in utero*, an impressive result that points to a central role of PTHrP in senescence.

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PHOSPHATE, KLOTHO, FGF23 AND MORE

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Ever since the discovery of FGF23 was announced at the 2000 ASBMR meeting, phosphate metabolism has held the meetings’ stage every year, and the 2005 Annual Meeting of ASBMR was no exception, as a startling new feature of FGF23 action was disclosed, and several other advances were also presented.

In an extraordinary poster presentation (50), the Klotho protein was shown to be a binding partner of FGF23 that is required for the action of FGF23 both *in vitro* and *in vivo*. Although *klotho* was originally described as a mutation that causes premature aging, *FGF23*(-/-) and *klotho* mice have essentially identical phenotypes - hyperphosphatemia and high $1,25(OH)_2D$ levels, with resultant hypercalcemia and progressive renal failure. However, in the *klotho* mouse, FGF23 levels are extremely high (50). Thus, an interaction between Klotho and FGF23 is required for either factor to be active with regard to phosphate or $1,25(OH)_2D$ metabolism. At about the same time as the 2005 ASBMR meeting, two papers reported related features of Klotho (51;52). One reported that Klotho increases surface display of the epithelial calcium channel TRPV5, thereby increasing renal calcium reabsorption, by cleaving sugars from the channel (51). The

other reported that overexpression of the *klotho* gene increases the longevity of mice, purportedly by inducing insulin resistance (52).

Collectively, these results place Klotho and FGF23 at the nexus of a new axis of vitamin D, calcium and phosphate homeostasis. FGF23 and Klotho are both regulated by vitamin D; they act as partners to increase phosphate excretion. Klotho also increases renal calcium reabsorption. Acting together, FGF23 and Klotho inhibit renal synthesis of $1,25(OH)_2D$. These actions may constitute a homeostatic system in which vitamin D increases calcium and phosphate absorption from the intestine and also, via Klotho and FGF23, promotes renal calcium retention and phosphate excretion, actions that would tend to absorb and retain calcium while rejecting phosphate. In this system $1,25(OH)_2D$ also feeds back on its own synthesis to regulate its level. In some undetermined fashion that could have to do with a link to carbohydrate metabolism, the Klotho/FGF23 system also regulates longevity. Altogether, it is a lot to digest, but details of the story were discussed in a *Commentary* in the November issue of BoneKEy (53).

It is increasingly clear that FGF23 responds to variations in phosphate intake. At this meeting, it was reported that dietary phosphate regulates serum FGF23 concentrations in healthy men (54), and a high phosphate meal induces a small increase in FGF23 after a lag of up to eight hours (55). Assay for the whole FGF23 molecule was found to be more sensitive than the standard carboxyl-terminal assay to changes induced by manipulating dietary phosphate (56). FGF23 levels were reported to rise after a hypoparathyroid state is induced by parathyroidectomy, with a lag of one to two days after the rise in serum phosphate (57). Changes in dietary phosphate were reported to increase FGF23 expression by osteoblasts, just as changes in the medium phosphate of cultured osteoblasts do (58).

X-linked hypophosphatemia in humans and the *Hyp* mutation in mice involve loss of the *PHEX/Phex* gene, which is highly expressed in bone cells. Several groups have concluded that *Phex* expression in bone is not responsible for phosphate wasting, because forced expression of *Phex* in bone cells does not fully rescue *Hyp* mice from phosphate wasting (59-61). However, use of *osteocalcin-cre* to remove *Phex* specifically from osteoblasts produces renal phosphate wasting, which is due to increased bone production and increased serum levels of FGF23 (62). Thus, bone is the physiologically relevant site of *Phex* expression. Several lines of evidence suggest that bone is also the physiologically relevant site of FGF23 expression, but that remains to be established firmly.

Hyp mice have hypophosphatemia and inappropriately low $1,25(OH)_2D$ levels: what is the relationship between these two features of the phenotype? Mice that overexpress the renal sodium-phosphate transporter NaPi-IIb and also carry the *Hyp* mutation have normal serum phosphate levels and normal production of $1,25(OH)_2D$, suggesting that abnormal renal phosphate handling in the proximal renal tubule is responsible for altering vitamin D metabolism (63).

A final surprise comes with the elucidation of yet another renal phosphate-wasting syndrome, hereditary hypophosphatemic rickets with hypercalciuria [OMIM 241530]. Affected members of a Bedouin kindred have loss-of-function mutations in the type IIc sodium-phosphate cotransporter NaPi-IIc, and several unrelated patients have an assortment of other mutations also predicted to prevent the function of this transporter (64). Presumably, altered renal phosphate handling as a consequence of the phosphate transporter mutation leads to an increase in the production of $1,25(OH)_2D$ and consequent hypercalciuria. What is surprising about this result is that NaPi-IIc is thought to comprise only about 10% of renal phosphate transporters and no human phosphate-wasting syndrome has yet been associated with a mutation in the more abundant transporter, NaPi-IIb.

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VITAMIN D RESEARCH

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Research presented at the annual meeting covered many facets of vitamin D biology, both old and new. The field is progressing well, but interestingly, appears to be revisiting certain fundamental issues which, surprisingly, have yet to be resolved. Significant incremental advances were reported in the basic sciences, as well as in the translational and clinical areas.

Basic Advances

The mechanism of action of vitamin D and its hormonal metabolites involves the vitamin D receptor (VDR), a transcription factor that acts at the level of the genome to modulate the output of target genes. Several studies focused upon the numerous details of this activity. We discovered that a process exists for the VDR's migration to the nucleus (65), and that this migration is influenced by phosphorylation of its partner RXR (66). Additional details include the ability of the receptor to bind to target genes in the absence of $1,25(OH)_2D_3$ and RXR (67), and the capacity of the protein to recruit transcription factor partners (68), as well as chromatin remodeling complexes, such as those containing the SWI/SNF enzymes (69). At the level of target genes, we learned about a surprising new participant in Cyp24 activation, namely the glucocorticoid receptor (70). We also gained an increased understanding of the molecular details of activation of target genes by $1,25(OH)_2D_3$, particularly as it relates to the calcium regulating channel TRPV6 (71). $1,25(OH)_2D_3$ also plays a role in the activation of RANKL gene expression via the chondrocytes (20). Mechanistically, new information has emerged regarding the ability of $1,25(OH)_2D_3$ to activate or modulate membrane pathways, including those of the JNK and p38 MAPK pathways (72;73) and to influence the canonical Wnt pathway (74).

We were also provided with new insights as to how the VDR is regulated, particularly with respect to calcium and PTH in kidney cells (75). While additional studies focused upon the impact of polymorphisms in the *VDR* gene on BMD and other skeletal consequences, particularly polymorphisms at the start site of translation (76), a new polymorphism observed in the *VDR* gene promoter appears to alter the expression levels of the VDR (77). Finally, several studies address the role of the VDR and its cognate ligand in osteoblastic versus adipocytic lineage determination (78), and the mechanism and role of PPARγ (79). Perhaps most fascinating was the discovery that knockdown of 1α-hydroxylase expression in cancer and other cells inhibits local production of $1,25(OH)_2D_3$ and thereby inhibits the capacity of $25(OH)D_3$ to prevent cancer cell growth both *in vitro* and following tumor cell transplantation in mice (80). This finding provides a much needed boost to the existence of extra-renal 1α-hydroxylation, at least in tumor cells. Additional studies confirm the role of the vitamin D binding protein in restricting the entry of $1,25(OH)_2D_3$ into cells, thus reducing the biological potency of the vitamin D hormone relative to those vitamin D analogs that do not interact with this serum carrier protein (81). This interaction may be instrumental in determining vitamin D analog potency. Collectively, these studies on basic mechanisms provide new insights into the molecular details of vitamin D action and the influence of extracellular components on those actions.

Vitamin D and the Osteoblast

The actions of vitamin D in bone are very complex, perhaps due to the role of the vitamin D hormone in the bone remodeling process. To complicate this further, the skeletal phenotype of VDR null mice is not particularly illuminating and is somewhat controversial. Be that as it may, it seems likely that the actions of $1,25(OH)_2D_3$ on osteoblast precursors, as well as on the osteoblast itself, are largely ones of proliferation and functional modulation rather than one of differentiation. In the latter case, the apparent capacity of $1,25(OH)_2D_3$ to downregulate sclerostin, and perhaps to influence the activity of the Wnt signaling pathway in osteoblast precursors (82), may

be a hint at things to come with regard to mechanism. This may be only part of vitamin D's action in osteoblasts, however, as new studies suggest that some of the activities of vitamin D are additive with those that result from Wnt activation (74). Regardless, it seems likely that the anabolic activities of vitamin D at the level of bone formation may reside in the hormone's capacity to modulate key bone anabolic pathways. Time will tell.

Animal Studies and Calcium and Phosphate Homeostasis

There is renewed interest in the individual roles of vitamin D and parathyroid hormone in skeletal mineral balance. These studies are being facilitated by the novel use of mouse strains in which either the *PTH* gene or the *1α-hydroxylase* gene, or both, have been deleted. Results suggest that both PTH and $1,25(OH)_2D_3$ exert unique actions that modulate calcium homeostasis at the level of osteoblasts and osteoclasts, and that their actions at the anabolic level are independent of one another (83). Similar studies also indicate that the $1,25(OH)_2D_3$ system, while not essential for the anabolic activities of PTH, is indeed required for the catabolic actions of this hormone (84). Genetically altered mice were also used to study the relationship between FGF23 and vitamin D metabolism (85). Additional studies were reported on the activities of vitamin D and its metabolites in modulating the expression of the lipoprotein ApoA1 (86).

Human Physiology and Therapy

A new focus is emerging on what constitutes normal levels of vitamin D, $25OHD_3$ and $1,25(OH)_2D_3$ in humans. This issue is yet unresolved due to issues of measuring vitamin D and its relevant metabolites in the blood (87-89) and in identifying individuals with so-called "normal" levels of vitamin D metabolites. The importance of this topic was addressed in an industry supported symposium on the role of vitamin D and its analogs in metabolism, cardiovascular health and long-term survival. This symposium stemmed from recent findings that highlight the cardiovascular protection and increased survival rates of patients with chronic kidney disease when taking certain vitamin D analogs.

Human Vitamin D Deficiency

Stemming from the above, a number of researchers are currently sounding an alarm that human populations in the United States, Europe, and elsewhere may be deficient in vitamin D. To some, this deficiency is viewed as perhaps epidemic in nature, highlighting the importance of resolving the real nature of this issue. Deficiency may be due, in part, to insufficient sunlight exposure (90) as well as to inadequate levels of vitamin D supplementation (91;92). It may also be due to additional factors such as liver disease (93). Vitamin D deficiency manifests itself in increased osteoporosis and thus in increased risk of fracture (92;94-98). Bone loss is also associated with other diseases such as inflammatory bowel (99) and celiac (100) disease, and may be associated with acute leukemia (101). We learn that there are additional consequences of vitamin D deficiencies, including increased hyperparathyroidism and its effects on bone (102-104) as well as effects on neuromuscular performance (105). Perhaps most importantly, there appears to be mounting evidence that large scale vitamin D deficiencies are leading to an increased risk of a wide variety of tumors, from colorectal to breast cancers. This apparent need to increase vitamin D intake, particularly through increased sunlight exposure, is currently clashing with the recommendation by other researchers to reduce sunlight exposure to lower the risk of various skin cancers.

Conclusions

Research advances at the recent meeting in the realm of vitamin D biology were significant. They are substantial contributions that increase our understanding of vitamin D, both at the level of its interesting molecular actions as well as its biological activities and therapeutic relevance. The increasing complexities of these actions of vitamin D suggest that while considerable progress has been made, new and exciting insights are also likely to emerge in this area in the near future.

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GENETICS OF OSTEOPOROSIS

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Mouse work continues to progress from QTL mapping for BMD and bone structure in crosses of various strains, to isolation of reasonably short (2-4 Mb) chromosomal regions in congenic mice (106), to identification of phenotype-modifying genes therein. Congenic mice carrying a chromosome 4 QTL for BMD first mapped in a B6 x DBA *2* cross present increased serum ALP levels, and higher indices of bone formation/mineralization *in vivo* (MAR) and *in vitro* (107). Sequence variants in the *Akp2* gene (also known as *Tnap*) in this region were compared between B6 and DBA2 mice, and a Pro324Leu variant in the protein sequence shown to modify ALP activity *in vitro*. By cleaving pyrophosphates, a major inhibitor of hydroxyapatite crystals, tissue non-specific ALP (TNAP) co-expressed with collagen Type I was recently shown to be necessary and sufficient for bone matrix mineralization (108). Since in humans TNSALP maps to 1p36, a major QTL for BMD in several linkage studies (109), these data strongly suggest that genetic variation in this gene might account for some of the variance in bone material properties in both mice and humans. The leptin receptor (*LEPR*) gene also maps on human chromosome 1p. Based on linkage and expression levels in rat bone, LEPR now appears as a possible candidate to explain differences in femur strength and geometry between rat strains (110). Moreover, the *fa/fa* rat model carrying an inactivating mutation of *LEPR* had bones with reduced cross-sectional size (110). These interesting observations may also help clarify the controversy about leptin's effects on the skeleton, since leptin inhibits trabecular bone modeling/remodeling by a central relay (111) but might have opposite (anabolic) effects directly on bone (112). Congenic mice carrying a chromosome 6 QTL first mapped in a B6 x C3H cross have decreased BMD, circulating and bone IGF-1, and increased body and bone marrow fat. In these mice expression of several fat genes (*PPARγ*, *Alox5* and *Klfl5*) was increased in bone (113), consistent with the notion that PPARγ reciprocally regulates osteogenesis and adipogenesis (114). Moreover, these congenic mice were resistant to metabolic and fat changes induced by a high fat diet, a finding similar to the Pro12Ala *PPARγ* mutation in humans, whereas the high fat diet increased bone mass in both congenic and B6 mice. How high fat may differentially affect metabolism and the skeleton in congenic mice remains to be elucidated.

Recently discovered mechanisms of regulating bone metabolism, including PPARγ (see above) as well as the Wnt/LRP5 (115;116) and adrenergic pathways (117), were further shown to contribute to variation in BMD and/or fracture risk in humans. Several intronic and one missense exonic SNPs in *PPARγ* were found to be significantly associated with spine and hip BMD, and calcaneum ultrasound properties, in a cohort of 740 men and 776 women, mean age 61 yrs (118). Polymorphisms Ala1330Val in LRP5, Ile1062Val in LRP6 and Arg324Gly in s-FRP3 each increased fracture risk nearly 50% in men (n=2686). Carriers of both LRP5 and LRP6 risk alleles had a 2.4 fold increased risk of fracture, whereas carriers of both LRP6 and sFRP-3 risk alleles had a decreased risk (119). In women (n=3900), these effects were less pronounced. In males, moreover, the LRP6 genotypes were associated with lumbar spine BMD and projected bone area, and with height, similar to the results of a previous association study with LRP5 variants (120). Wnt10b is an important molecular switch for the commitment of mesenchymal stem cells towards the osteoblastic and chondrocytic lineage at the expense of the adipocytic lineage (121). By sequencing the *Wnt10b* gene in Caucasians and Africans, 38 SNPs were identified, several of which were differentially expressed in these two ethnic groups (122). One of these SNPs, T>G in 3'-UTR, was associated with hip BMD in Caucasians (n=260) and Afro-Caribbeans (n=1001), although this polymorphism was much rarer in the latter, suggesting that

Wnt10b genetic variation might play a role in ethnic differences for bone mass. Two missense substitutions within the adrenergic receptor beta2 gene (*ADRB2*) and two synonymous SNPs at the *ADRB2* locus were reported to be associated with hip, and to a lesser extent spine, BMD in post-menopausal women, but not men (mean age 60 yrs) (123). Moreover there was an interaction between *ADRB2* SNPs and the use of beta-blockers on BMD, which directly supports the notion of an influence of beta2 adrenergic receptor-mediated signaling on bone mass in humans (124). An A163G polymorphism in the OPG promoter region, previously associated with fracture risk in humans (125), was confirmed to be associated with a nearly two-fold increase in fracture risk at the femur neck in a large prospective cohort of 6700 women (126). Contrary to other gene polymorphisms that appear to be more prominently associated with fracture risk than with BMD, such as Col1A1 and ESR1 genotypes (127;128), OPG-associated risk of fracture was largely dependent on BMD.

Although a number of twin and parent-offspring studies have clearly established that BMD, particularly peak bone mass, is highly heritable, heritability for bone loss and bone structure has not yet been extensively investigated in humans (129). Two studies, one in 261 females and 167 males from 18 extended Mexican-American families (130) and one in 176 peri- and post-menopausal twin pairs (131), both concluded that genetic factors may account for up to 40% of the variability in BMD changes. Some heritability estimates for bone architecture by pQCT at the tibia and radius were also presented in 8 large multi-generational Afro-Caribbean families (132). Interestingly, genetic effects on trabecular density were in the range of heritability for BMD (60-70%), whereas genetic effects on cortical bone were much lower (25-30%). The advent of 3D pQCT allowing *in vivo* high-resolution of trabecular micro-architecture in humans (133;134) represents a new opportunity to evaluate the contribution of genetic factors on the most discrete bone traits.

Several interesting discoveries were also reported concerning monogenic skeletal and metabolic disorders in humans. Mutations in carbonic anhydrase II, chloride channel gene (*CLCN7*), and vacuolar proton pump gene (*TCIRG1*) are responsible for various forms of osteopetrosis (135). LRP5 gain-of-function mutations have also been recently reported in Type 1 autosomal dominant osteopetrosis (ADO1) (136), which suggests that this disorder may better qualify as a sclerosing bone dysplasia. This notion was further supported by the evidence that osteoclasts (OC) isolated from ADOI patients are morphologically and functionally normal (137). Mutations in the *PLEKHM1* gene were found in an autosomal recessive form (intermediate type) of osteopetrosis (138). Starting from the (ia) rat model, the authors mapped the disease-causing gene to a 4.7 cM region that was subsequently sequenced. A deletion of one nucleotide in *PLEKHM1* was identified and later a splice site mutation in this gene was found in 1 family with the disease. The gene codes for a small GTPase which is widely expressed in rat tissues. Mutant osteoclasts are characterized by enlarged cytoplasmic vesicles, but the ultimate functional consequence of a *PLEKHM1* mutation on osteoclastic functions remains to be elucidated. *PHEX* and *FGF23* mutations are responsible for X-linked hypophosphatemic rickets (XLH) and autosomal dominant hypophosphatemic rickets (ADHR), respectively (139). FGF23 participates in phosphate homeostasis, for instance in response to diet (140), by regulating the renal expression of a sodium- phosphate co-transporter, NaPi-IIc. A 227delC mutation in the *NaPi-IIc* gene was found to cause hereditary hypophosphatemic rickets with hypercalciuria (64). The high serum calcium levels in this case result from an adequately increased synthesis of calcitriol ($1,25(OH)_2D_3$) in response to hypophosphatemia, in contrast to XLH and ADHR where the renal 1-α-hydroxylase is inhibited. Other mutations in *FGF23* that alter the processing and secretion of the molecule were reported to cause tumoral calcinosis with hyperphosphatemia (141;142).

Conflict of Interest: The author reports that no conflict of interest exists.

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IMAGING ASSESSMENT OF BONE

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For diagnosis, monitoring treatment response, and exploring the pathophysiology of osteoporosis and other metabolic bone disorders, advanced imaging techniques are essential because of advantages, such as their noninvasive or nondestructive nature and the capacity for 3D assessment. These techniques were well represented at the Annual Meeting. Quantitative assessment of volumetric bone mineral, structure, and biomechanical properties with computed tomography (CT), high-resolution CT, and micro CT has extensive application. As at previous ASBMR meetings, there were relatively fewer studies using MRI than CT, perhaps due to the complexity and associated high cost of MRI.

Vertebral CT

CT has been utilized for assessment of the human vertebral body, which is prone to osteoporotic fracture. Though DXA BMD is widely used to predict vertebral fracture risk, an approach incorporating better estimates of vertebral strength combined with spine loading in a population-based study with 375 women and 325 men has shown that 1) the age- and sex-differences in the 'factor of risk' mirror the reported prevalence of vertebral fracture, and 2) individuals at high risk for vertebral fracture have both lower vertebral strength and greater vertebral loads (143). Finite element models derived from QCT scans mechanically integrate all the geometrical and material property data within the scans to provide measures and predictions of vertebral strength. QCT BMD values of each bone voxel are converted into elastic modulus values using pre-determined correlation between the elastic modulus and QCT-derived BMD (144). A pilot study of 20 randomly selected post-menopausal women treated with PTH(1-84) for one year indicates that about half the overall increase in vertebral strength can be attributed to an average increase in bone density, and the remaining half of the effect is due to alterations in the distribution of bone density within the vertebra (145). The voxel size of 156 to 187 μm in-plane, and 300 to 500 μm through-plane, from vertebrae of osteoporotic women has demonstrated that differences in spatial resolution of the different CT scanners used had a significant influence on measured structural variables but did not affect longitudinal analyses (146). Long-term precision errors were 13% for bone volume fraction and 11% for trabecular thickness, corresponding to monitoring time intervals of 1 and 1.2 years. All measured structural variables in 67 patients treated with teriparatide at 20 μg/d for one year showed significant improvements. Increases were consistently larger during the first 6 months of treatment (147). The 2D trabecular parameters from 2D conventional radiograph demonstrated that hPTH(1-34) increases the ratio of trabecular bone/area, the interconnectivity index, and the trabecular network length (148).

Hip CT

The geometry of the proximal femur is much more complicated than that of the vertebral body. Trabecular structural parameters obtained from multidetector row CT in 4 femoral head specimens were highly correlated with their ultimate load, while the volumetric BMD and trabecular structural parameters obtained from multidetector row CT in the vertebral body of postmenopausal women revealed a strong association with prevalent vertebral fracture (149). CT examination shows that an increase in volumetric trabecular BMD at total hip and femoral neck in PTH-treated postmenopausal osteoporotic women (n=62) for 1.5 years was due primarily to an increase in volumetric trabecular BMC. Cortical bone volume increased significantly at both hip regions. The increase in volumetric cortical BMC was smaller than the increase in bone volume, and therefore there was a decrease in volumetric cortical BMD that was significant at the total hip, but not at the femoral neck (150). Helical volumetric quantitative CT examination of 14 crew members of the International Space Station demonstrated that total femur

integral BMC, but not integral volumetric BMD or trabecular BMD, recovered to its pre-flight value, 12 months after flights lasting 4-7 months. Recovery of bone mass involved increasing both bone density and bone size. Incomplete recovery of BMD in the hip in the year after long-duration spaceflight was observed. As shown by an increase in the minimum femoral neck cross-sectional area and integral tissue volume, the proximal femur appears to adapt to resumed load bearing by periosteal apposition (151). In 3158 men aged 65+ in the US who are enrolled in the Osteoporotic Fractures in Men Study (MrOS), the femoral neck and lumbar spine volumetric BMD was greatest in African Americans, while the femoral neck and lumbar spine cross-sectional area was lowest in African Americans and greatest in Caucasians, which might contribute to some of the ethnic difference in hip and vertebral fracture epidemiology (152).

Hip DXA and CT

The Hip Structure Analysis program to determine femoral neck BMD and cross-sectional geometry from DXA images has been used in male and female populations. Data from a population-based sample of elderly men from a town in Belgium suggested a predominant contribution of total and bioavailable estradiol rather than testosterone to the maintenance of the biomechanical competence at the femoral neck (153). It is unknown whether obesity makes fractures more likely or if fat padding reduces impacts in certain fractures. DXA femoral neck BMD and cross-sectional geometry in a substudy of 1410 women in the Women's Health Initiative Observational Study indicated that traumatic forces causing fractures are a function of body weight and that femurs of the obese are disproportionately weaker in relation to those forces (154).

Patient position during DXA scanning, limited resolution and limited signal-to-noise ratio of the DXA image, and actual 3D complicated anatomy are some of the factors that should be taken into consideration in interpreting the geometric parameters from 2D DXA image analysis of the hip, though DXA scanner resolution is less important in BMD analysis (155-158). For ideal geometry measurements, the resolution should be closer to 1 line pair/mm and should not vary with orientation of the bone in the image. Current commercial DXA scanners are less than ideal in this respect (155). A major limitation on DXA precision is that inconsistent femur position alters projected dimensions so that changes in time or differences between subjects cannot be distinguished from actual geometric differences. Precision by CT is considerably improved in all geometric parameters. The major advantage of higher precision is that research studies should be able to demonstrate significant geometric effects with smaller numbers of subjects than required for DXA (156). The cortical thickness can be as low as 0.5 mm at some portions of the femoral neck, which is below the spatial resolution available for *in vivo* CT imaging. The resulting partial volume averaging errors cause underestimation of cortical BMD and overestimation of cortical thickness (157). CT examination showed that the femoral neck cross-section is ellipsoid, and the ellipsoid size and orientation change along the axis. The circularity assumption used in the calculation of biomechanical properties from DXA can lead to large errors in estimating cross-sectional biomechanical properties, especially in the distal half of the femoral neck (158).

A study of 821 healthy Chinese (540 females) subjects and 1052 Caucasian (692 females) subjects aged 18-93 years and living in Melbourne showed that the femoral neck length was 6% shorter in Chinese subjects, compared to Caucasian subjects, after adjusting for their shorter stature. The disproportionately shorter femoral neck length in Chinese subjects contributes to higher hip strength and a lower hip fracture rate than in Caucasian subjects (159). CT-scan and biomechanical testing of the human femoral cortical bone at the midshaft showed that significant correlations were found between ultimate stress and CT-scan Hounsfield Units (160). The distal tibia CT examination of 440 children (225 boys, 215 girls) aged 9-11 years (Tanner stage I-III) showed that boys were significantly heavier

and had greater muscle cross-sectional area and that all bone geometric parameters were 5-15% greater for boys (161).

Micro CT

Micro CT has been used to examine microarchitecture of human specimens and the skeletal phenotype of rodents and various animal models. In animal studies, micro CT shows restoration of cancellous bone volume and connectivity in ovariectomized mice treated with basic fibroblast growth factor for a short term (3 weeks), which can be maintained following treatment with the anti-resorptive agent residronate for at least 5 weeks after withdrawal of basic fibroblast growth factor (162). A study in rabbits showed that micro CT can reproducibly quantify 3D microarchitecture of new bone formation inside the pores of the titanium prosthesis implants, which may find application in studying the effects of different sizes of pores and different coating materials on osteogenesis (163). Micro CT examination of rat tibia showed that PTH(1-34) effectively and dose-dependently increased osseointegration on titanium implants and enhanced implant anchorage in gonadectomy-induced low density trabecular bone (164). Micro CT showed that 3 signaling-selective PTH(1-34) analogs improved microstructure of trabecular bone in the distal femur, T5 vertebra, and femur mid-shaft cortical thickness in female mice (165). PTH(1-84) at 5, 10 or 25 μg/kg/day increased micro CT bone volume fraction with associated increases in trabecular number, thickness and connectivity density in the thoracic vertebrae of osteopenic rhesus monkeys (166).

Micro CT of human iliac crest biopsies (n = 8 /group) showed that compared to placebo-treated subjects, mean trabecular bone volume fraction was 45% higher in PTH(1-84)-treated subjects, with a 10% lower trabecular separation and a 55% lower structure model index, indicating a better connected trabecular architecture with a more plate-like structure, indicative of a stronger bone (167). Micro CT of longitudinal changes in 3D microarchitecture of paired iliac crest bone biopsies in postmenopausal women with osteoporotic vertebral fracture showed that 3D trabecular microstructure deteriorates in postmenopausal osteoporotic women without active treatment. Trabeculae shift from a plate-like structural type to a rod-like pattern, and become less connected. Unlike findings in 2D bone histomorphometry, 3D shows that trabecular thinning does occur in the patients (168). Hypoparathyroidism is a disorder in which PTH is absent from the circulation. Bone deficient in PTH is markedly abnormal. Increased bone density in hypoparathyroidism is associated with abnormal bone quality, increased 3D μCT bone volume fraction and trabecular thickness. Transform infrared spectroscopic imaging showed markedly increased crosslink ratios, consistent with increased mean bone age (169). Micro CT analysis of iliac crest biopsies from otherwise healthy premenopausal women who presented with an idiopathic osteoporosis and fragility fractures showed significant decreases in bone surface and increases in trabecular separation, suggesting abnormal bone remodeling associated with loss of whole trabecular elements, rather than thinning of individual trabeculae (170). Male normocalciuric idiopathic osteoporosis showed a low micro CT trabecular bone volume fraction and trabecular number, and a high trabecular separation in iliac crest biopsy (171). 2D bone histomorphometry of the transilial biopsy showed that the periosteal envelope of postmenopausal women forms bone at a lower rate than do the trabecular and endocortical envelopes (172). Histomorphometric data from the iliac crest suggest that alendronate positively affects cortical bone by permitting customary periosteal expansion, while slowing the rate of endocortical bone remodeling, an action that slows bone loss, from the endocortical surface (173). Logistic regression analysis demonstrated that trabecular number and cortical thickness on 2D histology of the iliac crest were the most positive variables to affect the risk of vertebral fracture (174). Finite element models from micro CT images of 13 human cadaveric thoracic vertebrae showed that the biomechanical role of the thin cortical shell in the vertebral body is substantial, and is maximal at the mid-section where the vertebra is narrowest.

The role of the trabecular bone can also be substantial and is greatest near the endplates. Densitometric assays of vertebral strength should include measures of both cortical and trabecular bone density. If trabecular bone density alone is to be monitored, the most relevant regions for sampling are near the endplates and not at the center, since it is precisely in the central section that the trabecular bone has the least influence on overall structural behavior (175).

Though not yet approved by the FDA, high resolution pQCT has been investigated in the US and Europe in human distal radius or tibia (176). No significant differences in bone architecture were found between radius and tibia in young healthy women (177). 3D pQCT of the radius, but not hip and spine DXA, could differentiate osteopenic women with and without a fracture history (178). A population-based study showed that relative to women, men begin adult life with a more plate-like, and thus stronger, trabecular microstructure, and that no change over life in trabecular number or separation occurs in men, as compared to significant decreases in trabecular number and increases in trabecular separation in women (133). 3D analysis of weight-bearing (distal tibia) and non weight-bearing (distal radius) sites suggests that Colle's fracture is mainly related to local cortical low mineral density instead of trabecular bone structure, whereas hip fractures are associated with a combination of both trabecular and cortical quantitative and qualitative damages occurring in both weight-bearing and non-weight-bearing bones (134).

MR Imaging

High field 9.4 Tesla MR microscopic imaging of ewes indicates that microarchitecture of trabecular bone contributes significantly to its biomechanical characteristics, independent of BMD measured in the femoral neck (179). In early postmenopausal women, MR images of the distal radius and tibia acquired at 137 x 137 x 410 μm^3 voxel size at baseline and 11-13 months showed significant structural changes (8-10%) in control subjects but little or no significant change in the HRT group (180). Gradient echo MR images showed that glucocorticoids alter trabecular architecture in the distal radius more than postmenopausal osteoporotic state (181).

One of the crucial parameters to assess bone quality is the degree of bone mineralization. Water and fat suppressed projection MR imaging was used to image the solid matrix content of rat bone specimens. The signal from the medullary cavity (water and fat) is largely suppressed, along with the water and fat signal inside bone tissues. This method provides a means to measure bone matrix density in small animals (182). To distinguish osteomalacia from normal bone, hypomineralization was induced in rabbits. 1H images were acquired at 400 MHz from excised cortical bone by 3D 1H solid-state imaging of the tibia at a resolution of 183 µm. Significantly, higher water content was found in animals with osteomalacia than controls. Thus, proton solid-state imaging of bone water can distinguish subtle differences in mineralization density and therefore may provide a new means for noninvasive assessment of degree of mineralization of bone (183).

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TREATMENT OF OSTEOPOROSIS

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The Need for Prompt Drug Therapy

Patients with prevalent and new incident fractures should be treated promptly because the risk of another fracture within 12 months is high. Lindsay *et al.* (184) report that of 7233 women developing a new vertebral fracture after their first fracture, 80% were left untreated. During 12 months follow-up, 1039 (18%) of untreated women had a vertebral or non-vertebral fracture. Of those treated, 1056 (15%) received treatment within 90 days of their fracture; 85 (8%) had a fracture (RR = 0.51 compared to no treatment). 358 (5%) received treatment

90 days after the fracture; 76 (21%) had a fracture.

Weycker *et al.* (185) report that among 16,031 women with osteoporosis started on treatment, 5% (724) had a fracture during follow-up. The risk was reduced by 50% in those with more than 180 days of therapy, compared with those receiving less than 30 days. Thus, most patients following fracture do not receive treatment. Among those that do, there was a risk reduction with a trend to fewer fractures in those receiving early rather than late treatment, and in those taking medication.

Poor Compliance, a Major Heath Problem

About 50% of subjects fail to take therapy within 12 months. Weekly preparations are better adhered to than daily preparations, but not by much. Whether monthly or yearly treatment will be better remains unknown. When factors associated with poor compliance are identified, they account for only a small proportion of the burden of poor compliance (186-191).

Poor compliance is associated with high fracture rates (192-194). Compliance with weekly therapy is better than daily therapy, but neither are above about 50% within 12 months (195-197).

Treatment Reduces Remodeling Quickly, and Early Fracture Risk Reduction

Two factors contribute to bone loss and structural decay: the negative bone balance in the basic multicellular unit (BMU) and the remodeling rate (activation frequency). As each of the many remodeling events removes only a tiny amount of bone from a surface, it is the high remodeling rate that drives bone loss eroding and thinning trabeculae, thinning the cortex and making it more porous. Remodeling suppressants reduce the remodeling rate. Recker *et al.* (198) reported that oral ibandronate reduces activation frequency. In the placebo, the median was 0.22 per yr (range of 0.11-0.39). With continuous or intermittent therapy, the values were halved; 0.1 (0.04-0.2) and 0.13 (0.08-0.2), respectively, and were comparable to pre-menopausal women. Gunther *et al.* (199) reported that 5 mg residronate reduced bone resorption markers within two weeks using 35 mg once weekly.

When an anti-resorptive is started, the many sites actively resorbing bone before the anti-resorptive was started complete the cycle by deposition of new bone that undergoes primary mineralization over 2-3 months, then secondary mineralization over 8-12 months. This may stabilize stress risers (eroded sites concentrating stress locally, producing microdamage) and contribute to the lesser incidence of fractures in treated subjects, relative to controls in whom fragility and fractures continue. BMD increases because of the filling of these remodeling sites. This increase in BMD is a function of the number of remodeling sites active before treatment and the potency and dose of anti-resorptive. The *same* drug given to patients with high remodeling will increase BMD (and reduce the remodeling markers) more than when given to patients with low remodeling. However, the risk reduction, relative to controls with high or low remodeling, respectively, will be the same.

A lesser change in BMD does mean the fracture risk reduction will be less. Recker *et al.* (200) report that among 1423 women treated for 312 ± 254 days, fewer women taking raloxifene 60 mg/d had vertebral fractures than those taking alendronate 10 mg/d; 5(1.9%) v 8(3.1%). The sample size was small and there were few fractures, making interpretation difficult. There was no difference in the total number of fractures, numbers of vertebral, non-vertebral or hip fractures.

Is Fracture Risk Reduced in People Who Lose Bone During Therapy?

The completion of remodeling, stabilization of stress risers, and increase in tissue mineral density partly restores bone strength. At the new steady state where the slower birth rate of BMU and closure rate of completed BMUs is the same, bone loss is likely to continue if there is a negative BMU balance within each of the now few foci remodeling bone surfaces. However, the loss of tissue is likely to be very small,

because the remodeling rate is suppressed and the balance in the BMU is probably less negative because the osteoclasts resorb less bone. The more shallow and fewer resorption pits may produce cortical and trabecular thinning, but the chance of perforation of a trabecula is less, so it is likely that the risk of fracture remains reduced relative to controls even though bone loss is continuing. Watts *et al.* (201) report that fracture risk reduction using residronate was no different in those that gained bone and those that lost. The fracture risk reduction was around 40%, irrespective of whether the change in bone density (gain or loss) was at the spine or femur.

Continued treatment maintains the reduced remodeling, but remodeling removes micro-damage, so if reduced there may be counterproductive accumulation of microdamage. Increases in tissue mineral density may increase production and extension of micro-damage, as reported in animals using high doses of bisphosphonates. Allen *et al.* (202) report suppression of remodeling and increased microdamage in beagles after 12 months. Activation frequency was dose dependently suppressed with residronate and with alendronate. Trabecular crack density did not differ by drug but was 3-4 times higher than in controls. Higher doses increased crack density 5-fold. Both drugs increased vertebral stiffness with no other effects on mechanical properties. The single similar study in humans by Stepan *et al.* had small sample sizes and is difficult to interpret (203). What is clear is that bone loss resumes when treatment is stopped (204;205).

If periosteal apposition is partly a compensatory response to endosteal bone loss, then drugs that inhibit endosteal resorption should inhibit periosteal apposition. Bare *et al.* (173;206) report that hormone replacement therapy reduced the percentage of women with double label on periosteal and endocortical envelopes while alendronate reduced formation on the endocortical envelope only. Iwata (207) reports that 6 month female rats given residronate 0.05 µg/kg or .5 µg/kg subcutaneously daily for 17 days, or alendronate at 0.1 µg/kg and 1 µg/kg or 10 µg/kg, reduced periosteal apposition in the femur and tibia.

Anti-Resorptives and PTH

Prior use of alendronate delays the response to PTH. This effect seems to be temporary. Cosman *et al.* (208) report that responses to rechallenge with PTH(1-34) at 25 mcg/d 12 months after initial PTH treatment and alendronate were similar to the response obtained initially, suggesting that ongoing alendronate does not impair rechallenge with PTH to stimulate bone formation markers. Gasser *et al.* (209) report normal response to PTH following acute intravenous alendronate or zolendronate, but delayed responses with 16 weeks of alendronate in 16 month old rats treated with alendronate before PTH (100 µg/kg/5 times per week). Single iv alendronate 200 µg/kg or zolendronate at either 32 µg/kg or 320 µg/kg did not blunt the response to PTH. The reasons for the delay in anabolic response are unknown.

Preventing Resorption but Allowing Bone Formation to Continue

The mechanisms responsible for the reported fracture risk reduction with strontium ranelate are not understood. There is no compelling evidence that this is an anabolic agent. Whether it reduces resorption while allowing bone formation to continue is unresolved. Histomorphometric data were reported by Arlot *et al.* (210) using biopsies obtained at baseline and years 1-5, using pooled data from SR treated patients (n = 49) and pooled data from placebo plus baseline in the SR treated group (n = 87). Higher osteoblast surfaces were reported (38%, $p < 0.05$), as were greater appositional rates of 8% and 11% in cancellous and cortical bone, with no change in activation frequency and a trend toward lower endosteal and cancellous osteoclast surfaces and osteoclast numbers. Cancellous osteoid surfaces were lower, while mineral appositional rate was higher, in treated subjects with no change in osteoid volume and mineralization lag time.

Cathepsin K inhibitors prevent bone resorption. Periosteal apposition may be stimulated as well. Kumar *et al.* (211) used SB462795 in male monkeys (3, 30 or 1,000 mg/kg/d for 12 months) and reported a 30-70% reduction in resorption markers with an increase in BMD, but also a contraction in mid-femur medullary cavity area, suggesting net bone formation. The authors also suggested there may be an increase in periosteal bone formation with 40-50% increases in vertebral tolerated loads. Stroup *et al.* (212) studied ovariectomized monkeys treated with SB462795 at 1, 3, and 10 mg/kg/d, vehicle or alendronate at 0.05 mg/kg/IV for 9 months, finding an earlier reduction in resorption markers than with alendronate, with a lesser decrease in formation markers, but an increase in serum osteocalcin and a 150-200% increase in periosteal bone formation relative to ovariectomized controls. Endosteal porosity and bone formation rate was higher than in the oophorectomy control using the lowest dose. Similar anti-resorptive effects were reported *in vitro* and *in vivo* using the cathepsin K inhibitor AAE581 (213).

Henriksen *et al.* (214) report that inhibition of osteoclastic bone resorption *in vivo* by blocking acidification reduces bone resorption, but not bone formation. The acidification attenuates osteoclasts' resorbing activity, but these cells may still generate signals essential for bone formation. The inhibition of resorptive activity was achieved by the release of calcium that occurs during acidification of the resorption site and produces apoptosis of osteoclasts.

Li *et al.* (215) report that 3 month old male rats treated with OPG 10 mg/kg/twice weekly after gonadectomy had 5% greater periosteal and 8% greater endocortical diameter than controls, producing a 16% increase in CSMI. OPG caused a 33% increase in periosteal bone formation rate and a 142% reduction in endocortical bone formation rate. Placebo treated animals had a 9% and 12% decrease in periosteal and endocortical diameters, resulting in a 27% decrease in CSMI. Femoral shaft periosteal bone formation rate was decreased by 38% and endocortical bone formation rate was increased by 133%. In orchidectomised placebo-treated animals, there was a 67% reduction in bone volume at the distal femur, while in OPG-treated animals osteoclast surfaces were decreased by 67%, and bone volume increased by 107%.

Other Steps in Resorption Inhibition

Hannon *et al.* (216) assessed the effect of AZD0530, a selective inhibitor of Src kinase that is involved in osteoclast activation and an inhibitor of Ab1 kinase involved in osteoblast function. Treatment in 13 healthy, male volunteers, at a range of doses, suppressed bone resorption markers reversibly with variable changes in bone formation markers.

Atkinson *et al.* (217) report that AMG162, a human monoclonal antibody that inhibits bone resorption by neutralizing RANKL, was assessed in male monkeys treated with 50 mg/kg/m for 12 months. Femoral diaphysis cortical area increased (26%), as did bending strength (40%). At the lumbar vertebra, BMC and BMD increased by about 40-45%. This was associated with a 101% increase in compressive load, a 97% increase in apparent strength, and a 78% increase in toughness.

Blocking Inhibitors of Bone Formation

Sclerostin inhibits osteoblast production. Its absence should increase bone formation. Warmington *et al.* (218) report that sclerostin monoclonal antibody reverses oophorectomy-induced bone loss. Six month old rats were oophorectomised and allowed to lose bone for 12 months. Treatment for five weeks produced an increase in BMD of 26% at the spine, 16% at the whole leg, 28% at the femoral metaphysis, and 9% at the femoral diaphysis. Bone volume increased by 118%, BMD by 64%, trabecular number by 50%, trabecular thickness by 57%, and cortical thickness by 26%, and endosteal circumference decreased by 14%. TRAP-5B, a resorption marker, was deceased by 43% relative to controls.

Conflict of Interest: The author reports that he is an advisory committee member for Sanofi-Aventis, Eli Lilly, Merck Sharp & Dohme, Novartis, and Servier, and that

he lectures occasionally at conference symposia for those companies.

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