Growth Plate, Bone and Calcium

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Growth plate and bone are highly organized structures with a complex cellular biology. Studies have been limited by the relative lack of relevant experimental models. Thanks to new technologies, e.g. targeted deletions of genes, the knowledge is rapidly expanding. The first paper selected representing the **mechanism of the year** describes a new cause of autosomal recessive hypophosphatemia. Under **new paradigms**, we chose two papers showing that FSH directly regulates bone mass. The possibility to transplant mesenchymal stem cells and/or autologous tissue-engineered composites gives **new hope** for the therapeutic intervention of growth plate injuries. Another new hope comes from a report of growth plate-sparing effects of a new selective glucocorticoid receptor modulator. A **new concern** is the increasing amount of reports of bisphosphonate-induced osteonecrosis of the jaws. **Concepts revised or re-centered** are represented by a paper suggesting that the skeletal benefits from calcium supplementation are limited. **Important observation for clinical practice** is represented by a report of reduced bone mineral density and increased bone metabolism in young adults with 21-hydroxylase deficiency. A paper by Kollet et al. was selected to describe a **new mechanism** of osteoclasts to be involved in the mobilization of hematopoietic progenitor cells. In the section **new genes**, a paper identifying a new cause of recessive lethal osteogenesis imperfecta is discussed. The **review** of the year entitled 'C-type natriuretic peptide (CNP) in growth: A new paradigm' elegantly discusses the critical role of CNP in the regulation of linear bone growth not only in mice but also in humans. Finally, we selected two papers supplying some **food for thought**.

Mechanism of the year

Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism

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Background: Dentin matrix protein 1 (DMP1) is highly expressed in osteocytes and plays an important role in bone remodeling. Knockout mice for DMP1 have hypomineralized bones. Dmp1 null mice and humans with autosomal recessive hypophosphatemic rickets have rickets and osteomalacia with isolated renal phosphate wasting, associated elevated fibroblast growth factor-23 (FGF23) levels and normocalciuria. Thus the authors investigated whether this gene regulates bone mineralization not only directly but also through the regulation of phosphate homeostasis.

Results: Mutational analyses in two families with autosomal recessive hypophosphatemic rickets revealed two different mutations in the DMP1 gene. One mutation affected the start codon, and the other was a 7-bp deletion disrupting the highly conserved DMP1 C-terminus. Studies in DMP1 null mice demonstrated defective osteocyte maturation and increased FGF23 expression leading to pathological changes in bone mineralization.

Conclusion: These results suggest that a bone-renal axis is necessary for proper bone mineral metabolism.

DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis

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Background: Hypophosphatemic rickets is a genetically heterogeneous disease.

Results: The autosomal recessive form was mapped to chromosome 4q21. In addition, homozygous mutations in DMP1 (dentin matrix protein 1) were identified, encoding a non-collagenous bone matrix protein expressed in osteoblasts and osteocytes. Furthermore, the phosphaturic protein FGF23 was elevated in the plasma of 2 of 4 affected individuals.

Conclusion: The data provide a possible explanation for the phosphaturia and inappropriately normal $1\alpha,25\text{-dihydroxyvitamin D}_3$ [$1,25(\text{OH})_2\text{D}_3$] levels and suggest that DMP1 may regulate FGF23 expression.

Over the last years a big progress has been made in our understanding of the molecular basis of the different forms of hypophosphatemic rickets. The common denominator has been FGF23, a protein with phosphaturic action. The enzyme PheX inactivates FGF23 by cleavage. In X-linked hypophosphatemic rickets the PheX gene is mutated (inactive), whereas in the autosomal dominant form, FGF23 is mutated in the PheX cleavage site. These two articles clarify the etiology of autosomal recessive hypophosphatemic rickets (ARHR). Both research groups identified mutations in dental matrix acidic phosphoprotein 1 (DMP1) gene in patients with ARHR. The mechanism how DMP1, a bone matrix protein involved in mineralization, causes hypophosphatemia is still unknown. From these studies, PHEX does not seem to be involved. More interestingly, as in X-linked hypophosphatemic rickets, many patients have elevated serum levels of FGF23 or in the upper normal range. Furthermore, the paper by Feng et al. suggests that osteocyte has an important functional role in regulating mineral metabolism. Despite big steps in our understanding of phosphorus homeostasis in recent last years, much still remains unknown. The treatment of hypophosphatemic rickets is not optimal, with side effects, and often linear growth does not improve. A better understanding of phosphorus homeostasis and related pathways could help us to develop a better treatment in the future. The amazing message is that it is the bone that through several mechanisms coordinates systemic phosphate balance. After all, the bone contains 85% of all body phosphate.

New paradigms

FSH directly regulates bone mass

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Background: Postmenopausal osteoporosis is traditionally explained by decreased estradiol levels due to a decline in ovarian function. However, at the same time, FSH (follicle-stimulating hormone) levels increase dramatically. It has previously been shown that while ovariectomy itself induces bone loss, the response is blunted in hypophysectomized animals. This suggests that FSH may directly affect bone metabolism.

Methods: Measurements of bone mineral density and bone histomorphometry were performed in FSH receptor and FSH null mice. Serum FSH was measured by RIA and serum TRAP and osteocalcin by ELISAs. Osteoclastogenesis of mouse bone marrow cells, macrophages and human monocytes was studied in the presence of FSH. The resorptive activity of human monocyte-derived osteoclasts was detected by number and size of resorption pits formed on dentine. The effect of FSH on the formation of osteoblastic cells was also examined.

Results: Hypogonadal FSHR and FSH null mice were surprisingly observed not to lose bone and heterozygotic FSH^{+/-} mice even had a slight increase in BMD. FSH receptors were expressed on the osteoclast surface and FSH increased osteoclast differentiation and bone resorption in vitro. FSH signaling in osteoclasts was mediated via the MEK/Erk, NF- κ B and Akt pathways. However, FSH did not have any effect on osteoblasts.

Conclusion: FSH stimulates the formation and function of osteoclasts in vitro and in vivo. Despite the normal estrogen levels, haploinsufficiency of circulating FSH increases bone mass by reducing osteoclastic bone resorption. This indicates a direct estrogen-independent action of FSH on bone metabolism.

Follicle-stimulating hormone stimulates TNF production from immune cells to enhance osteoblast and osteoclast formation

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Background: It is well known that declining estrogen production after menopause causes osteoporosis in which the bone resorption exceeds the increase in bone formation, thus leading to net bone loss. However, a recent study suggests that FSH would also be an important direct, estrogen-independent regulator of bone mass. This study aimed at studying the possible involvement of TNF- α and ascorbic acid in FSH-induced bone loss.

Methods: TNF- α levels in FSH-deficient mice were measured by ELISA. TNF- α production was studied in bone marrow granulocytes and macrophages in vitro and different cell populations were identified by flow cytometry. A mathematical model of bone metabolism was used to understand how elevations in TNF- α contributed to high-turnover bone loss. Osteoclast and osteoblast formation assays were performed in the presence of TNF- α and ascorbic acid.

Results: FSH-deficient mice were shown to have low circulating levels of TNF- α compared to littermate controls. In parallel, the exposure of bone marrow cells to recombinant FSH caused an increase in TNF- α secretion. Macrophages and granulocytes were the main cell populations responsible for this effect. By applying a mathematical model and in vitro studies, it was concluded that the TNF- α -induced increase in osteoclast number accounted for most of the observed high-turnover bone loss. Interestingly, ascorbic acid reversed the stimulatory action of TNF- α on osteoclast precursors.

Conclusion: The reported effects of FSH on bone mass are partially exerted via TNF- α production by bone marrow macrophages and granulocytes. Furthermore, ascorbic acid may prevent FSH-induced bone loss in hypogonadal states by modulating the pro-resorptive actions of TNF- α .

These two papers suggest a new role for follicle-stimulating hormone (FSH) in the central regulation of bone mass. The authors report observations from which they conclude that high circulating FSH levels, and not declining estrogen levels, would be responsible for the bone loss observed in hypogonadal states, and that this bone loss would be partially mediated by immune cell-derived TNF- α . In the report by Sun et al., the deletion of FSH receptors (FSHR) had unexpectedly no effect on bone mass despite the presence of low serum estrogen levels. Surprisingly, also in the absence of FSH, there was no bone loss and even some gain. The most intriguing finding was that FSH heterozygous mice, despite having normal estrogen levels and 50% reduction in FSH levels, had decreased bone resorption. Half a year later, the same group published new data partially in the same animals. This time, Iqbal et al. focused on the possible involvement of TNF- α and immune cells in FSH-induced bone loss. They demonstrated that FSH null mice, despite low estrogen levels, have unexpectedly low levels of TNF- α . They furthermore showed that FSH stimulates TNF- α secretion from macrophages and granulocytes in vitro. A mathematical model was used to demonstrate that TNF- α actions mainly target osteoclast precursors and in vitro studies were used to confirm that this stimulatory effect was reversed by ascorbic acid, suggesting that vitamin C supplementation might be useful in the treatment of postmenopausal osteoporosis. Both reports have been criticized by some scientists working within the bone field [1–3]. Their main criticism focused on lack of data on circulating levels of estrogen and especially testosterone in these animals. It has previously been shown that FSHR null mice have high levels of testosterone, and thus it is possible that testosterone itself or its further aromatization

Fig. 1. Novel paradigm of hormonal control of bone mass. Under decreased gonadal function (menopause), estradiol levels are decreased and FSH levels are increased. This leads to increased activity of osteoclasts, both directly and indirectly via increased TNF- α production by immune cells.

into estrogen is sufficient to prevent bone loss in these animals. Thus at this point it may be better to conclude that both estrogen and FSH are important in regulating the bone response to menopause and gonadectomy as outlined in figure 1. Most likely, further studies with proper measurement of sex steroids and LH, as well as studies on combined effects of FSH with anti-estrogens, androgen receptor blockers or aromatase inhibitors are needed before this new mechanism will be generally accepted. However, the paper by Iqbal et al. brings a new nutritional aspect for the treatment of postmenopausal osteoporosis. They demonstrated that ascorbic acid (vitamin C) reversed the actions of TNF- α on both osteoclasts and osteoblasts, suggesting that supplementation with vitamin C might be a beneficial tool for the prevention of low bone mass and risk of fractures.

New hope

RANKL inhibition: a novel strategy to decrease femoral head deformity after ischemic osteonecrosis

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Background: Legg-Calve-Perthes disease is a juvenile form of osteonecrosis of the femoral head which can produce permanent femoral head deformity and premature osteoarthritis. Femoral head deformity is characterized by a predominance of bone resorption that produces a fragmented appearance and finally

collapse of the femoral head. Inhibition of interaction between RANK and RANKL by osteoprotegerin (OPG-Fc) is a potential therapeutic strategy to prevent this condition.

Methods: Ischemic osteonecrosis was surgically induced in 18 male piglets by disrupting the blood flow to the right femoral head. Two weeks later, OPG-Fc or saline was administered subcutaneously to 9 animals per group for 6 weeks. The left femoral heads from the animals treated with saline served as normal, non-disease controls. At 8 weeks, radiographic, histomorphometric, and immunohistochemical analyses were performed.

Results: Radiography showed significantly better preservation of the femoral head structure and higher ratio of epiphyseal height to diameter in the OPG-Fc group compared with the saline group. Histomorphometry revealed a significant reduction in the number of osteoclasts present in the treated group, and trabecular bone parameters (volume, number and separation) were significantly better preserved in the OPG-Fc group. No significant difference in femoral length was observed. By immunohistochemistry, OPG-Fc was observed only within the blood vessels, with no apparent staining of bone matrix or trabecular bone surfaces.

Conclusion: RANKL inhibition decreases bone resorption and femoral head deformity after ischemic osteonecrosis. Because RANKL inhibitors, such as OPG-Fc, do not bind to bone, their effects on resorption are reversible. This is very appealing when treating pediatric bone diseases such as Legg-Calve-Perthes disease, where the resorptive stage of the disease lasts for 1–2 years.

Interactions between osteoblasts and osteoclasts via the RANK-RANKL-OPG system play a major role in bone metabolism. This paper describes a new treatment strategy of ischemic femoral head deformity (Perthes disease) based on the inhibition of RANKL. The authors report for the first time that RANK-RANKL interaction plays a role in pathological bone resorption in femoral head deformity. This interaction can be targeted by soluble RANKL inhibitor and thus there exists a possibility to preserve femoral head and epiphyseal trabecular bone after ischemic osteonecrosis. Previous animal studies have suggested that bisphosphonates would be the treatment of choice to decrease femoral head deformity and prevent disease progression. However, the long-term effects of bisphosphonates on the growing skeleton are not known. Thus, for pediatric conditions, the reversible nature of RANKL inhibition as a therapeutic strategy is appealing. However, this study has some limitations. Only the short-term results were analyzed and it is not clear whether the effects will prevail. Furthermore, to counteract the effects of porcine-derived neutralizing antibodies against human OPG-Fc, the dosing in this study was two times higher than in a previous clinical study [4]. More importantly, it is well known that RANK-RANKL interaction also plays a role in regulating T-cell/dendritic cell communications and lymph node formation [for review, see 5]. It is thus possible that RANKL inhibition may have some immunological consequences and thus detailed investigations on possible side effects are needed before this new treatment strategy can be used in clinical practice.

Orally bioavailable GSK-3 α/β dual inhibitor increases markers of cellular differentiation in vitro and bone mass in vivo

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Background: Glycogen synthase kinase-3 (GSK-3) is a component of canonical Wnt signaling. Inactivation of GSK-3 leads to stabilization, accumulation and translocation of β -catenin into the nucleus and further activation of downstream Wnt genes. The effects of the small molecule inhibitor of GSK-3 (603281-31-8) on pre-osteoblastic cells and osteopenic rats were studied.

Methods: Mouse mesenchymal cells were treated with GSK-3 inhibitor and assayed for β -catenin levels, Wnt-responsive promoter activation, as well as for osteogenic and adipogenic markers. In vivo, GSK-3 inhibitor was administered daily for 60 days to ovariectomized rats. Treatment was started 1 month after ovariectomy. At the end of treatment, bone mineral density, bone histomorphometry, biomechanical strength and the expression of osteoblast-related genes were measured.

Results: Treatment of mouse mesenchymal cells with GSK-3 inhibitor increased the levels of β -catenin followed by Wnt-responsive promoter activation. Markers for osteogenic differentiation (bone sialoprotein,

collagens type I and V, osteocalcin, alkaline phosphatase and runx2) were upregulated. Ovariectomized rats treated with GSK-3 inhibitor had significant increases in bone mineral content and bone mineral density. This was associated with increased bone strength. Histomorphometric analysis revealed increased bone formation but no effect on bone resorption. The mRNA levels for collagens, biglycan, osteonectin and runx2 increased in response to GSK-3 inhibition in rat femur.

Conclusion: The GSK-3 inhibitor is capable of inducing osteoblast differentiation and increasing bone formation markers in vitro. After oral administration to ovariectomized rats, this small molecule increased markers for bone formation and improved bone mass and strength.

Besides many morphogenic growth factor- and hormone-signaling pathways, the canonical wingless (Wnt) pathway plays an essential role in the development of the skeleton and formation of bone tissue. In mesenchymal progenitor cells, the Wnt-signaling pathway is critical for the differentiation of the progenitors into osteoblasts and chondrocytes. Wnt functions through a co-receptor (LRP-5) and Frizzled receptor that transduce the signal through either the canonical β -catenin pathway or the non-canonical pathway. Following the discovery that Wnt/LRP-5/ β -catenin signaling is anabolic for bone, there have been continuous efforts in finding new anabolic agents acting on this pathway. One potential target is GSK-3, an enzyme that is a part of the β -catenin inactivation complex. Lithium chloride has previously been demonstrated to inhibit GSK-3 and thus increase bone mass [6]. Here the authors demonstrate that GSK-3 inhibition increased the levels of β -catenin and activated Wnt signaling in stem cell-like pluripotent cells. This was followed by a decrease in proliferation, a biphasic increase in osteogenic markers and a decrease in adipogenic markers. This indicates that GSK-3 inhibition predisposes mesenchymal stem cells to osteoblast commitment instead of adipogenic differentiation, thus acting in a 'pro-bone, anti-fat' way. The most interesting results in this study were obtained with ovariectomized rats fed with the GSK-3 inhibitor, where the GSK-3 inhibition maintained bone mineral density and improved bone microarchitecture. This suggests that GSK-3 inhibitors are new promising therapeutic compounds that, in the future, may be available for the treatment of osteoporosis and other states of increased bone loss.

The growth plate-sparing effects of the selective glucocorticoid receptor modulator, AL-438

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Background: It is well known that long-term use of glucocorticoids can cause growth retardation in children, an effect mediated through their actions on growth plate chondrocytes. The non-steroidal anti-inflammatory agent AL-438 acts through the glucocorticoid receptor and is known to retain full anti-inflammatory efficacy while having reduced negative effects on osteoblasts compared to those elicited by prednisolone or dexamethasone.

Methods: The murine chondrogenic ATDC5 cell line was used to compare the effects of AL-438 with those of dexamethasone and prednisolone on chondrocyte dynamics. The effects on bone growth were evaluated in cultured fetal mouse metatarsal bones.

Results: Dexamethasone and prednisolone caused a reduction in cell proliferation and proteoglycan synthesis, whereas exposure to AL-438 had no effect. Fetal mouse metatarsals grown in the presence of dexamethasone were shorter than control bones whereas AL-438-treated metatarsals paralleled control bone growth.

Conclusion: The data suggest that AL-438 has a reduced side effect profile on chondrocytes and bone growth compared to other glucocorticoids. This could prove important in the search for new anti-inflammatory treatments for children.

Glucocorticoids are the most effective anti-inflammatory agents known. However, in children, their long-term use leads to growth retardation through a combination of glucocorticoid-mediated effects on the systemic GH/IGF-1 axis, negative calcium metabolism, as well as direct effects on the growth plate. The search for a selective glucocorticoid receptor modulator that has the anti-inflammatory properties of conventional steroids without one or more of the side effects has been a longstanding

goal. The mechanism of action of the non-steroidal anti-inflammatory agent AL-438 is based on the hypothesis that many genes involved in undesirable side effects are upregulated (such as enzymes in lipid and muscle metabolism), whereas many proinflammatory genes (i.e. IL-1, IL-6 and TNF- α) are repressed. The authors used a well-established model of cultured fetal mouse metatarsals and were able to demonstrate that bones treated with AL-438 grow parallel to control bones while dexamethasone-treated bones were shorter. Furthermore, the authors confirmed that AL-438 maintains a similar anti-inflammatory efficacy as dexamethasone which was important and proved that AL-438 was still fully efficacious as an anti-inflammatory agent. Thereby the 'proof of principle' exists, i.e. that a non-steroidal anti-inflammatory agent is capable to selectively maintain its anti-inflammatory effects without negatively affecting bone growth. However, it should be emphasized that this conclusion is based on results from in vitro studies in mouse tissues. Therefore, it still remains to be shown if this approach will work in real life.

Treatment of rabbit growth plate injuries with an autologous tissue-engineered composite

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Background: Growth plate injuries could potentially be treated by tissue engineering. The authors attempted to investigate if it is possible to use autologous tissue-engineered composites in the treatment of growth plate injuries.

Methods: Chondrocytes were obtained from iliac crest epiphyseal growth plate cartilage taken from immature New Zealand rabbits. After proliferating in monolayer culture in vitro for 3 weeks, the cells were harvested and seeded onto a demineralized bone matrix scaffold to construct an autologous tissue-engineered composite. The composites were implanted into a defect in the proximal right tibiae growth plate created in 12 rabbits (group A underwent the operation after obtaining chondrocytes 3 weeks beforehand), another 12 rabbits were implanted with only the demineralized bone matrix scaffold (group B), and the defects in group C (12 rabbits) were not implanted. The left tibias of all animals were left undone as the normal control.

Results: When examined by x-ray 2 weeks after the operation, severe shortness and angulation deformity of the right tibia were gradually observed in groups B and C. However, there were no obvious changes in group A and there were significant differences between group A and groups B and C after 4, 8, and 16 weeks. Histological examination after 16 weeks revealed that the defects of the right tibias in group A had restored to almost the normal growth plate structure.

Conclusion: Tissue-engineered composites established by the combination of autologous growth plate chondrocytes and demineralized bone matrix has the potential to be used to prevent the formation of bone bridges and restore growth after growth plate injuries.

Repair of full-thickness cartilage defects with cells of different origin in a rabbit model

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Background: The purpose was to evaluate the repaired tissues formed in full-thickness cartilage defects in a rabbit model implanted with four types of chondrogenic cells.

Methods: Articular derived chondrocytes, mesenchymal stem cells (MSCs), and fibroblasts were isolated from 6-week-old New Zealand rabbits, and human umbilical cord blood stem cells were isolated from the umbilical cord blood of newborn children. The cells were cultured in vitro and embedded in polylactic acid matrices. Full-thickness defects were produced in the femoral trochlear grooves of both knees in adult rabbits. Cell/polylactic acid composites were transplanted into the cartilage defects.

Results: Full-thickness cartilage defects treated with chondrocyte or MSC transplantation were repaired with hyaline-like cartilage tissue, and repair was significantly better than in tissues treated with fibroblasts

and human umbilical cord blood stem cells, as well as in the control group. Repaired tissues treated with MSCs had better cell arrangement, subchondral bone remodeling, and integration with surrounding cartilage than did repaired tissues generated by chondrocyte implantation.

Conclusion: MSCs might be the most suitable cell source for cartilage repair. Further investigation into human umbilical cord blood stem cell transplantation is needed. In this study performed in rabbits, MSCs supplied the most promising cell source for cartilage repair.

The shortening and angulation of limbs caused by the bone bridge between the epiphysis and metaphysis is one of the big problems encountered by many orthopedists. Traditional methods, such as bone bridge cutting and fat packing, have many shortcomings. Progress in cell biology and biomaterial technology has led to the possibility of therapeutic applications of tissue engineering for the repair of cartilage defects. In the paper by Jin et al., an attempt was made to treat long bone growth plate injuries in rabbits by using an autologous tissue-engineered composite established in vitro. Although complete prevention of an angular and shortening deformity was not possible, the study demonstrates the potential of the method in treating growth plate injuries. It is important to emphasize that the graft can only efficiently prevent bone bridge formation in acutely injured rabbit models. The second paper by Yan et al. aimed to compare different cell sources for their potential to repair growth plate defects. Full-thickness cartilage defects treated with articular chondrocytes or MSC transplantation were repaired with hyaline-like cartilage tissue, and repair was significantly better than in tissues treated with fibroblasts and human umbilical cord blood stem cells. Damaged growth plates repaired with MSCs had the best histomorphology and integration with surrounding cartilage. The evaluation was pure histological and the follow-up time was only 12 weeks, a time-point when many patients have not yet been diagnosed with growth plate injuries. Although the data in both these reports are promising, there still exists a big challenge to establish a treatment protocol for patients who have suffered from growth plate injuries over a long period of time and obviously already have developed a deformity of the limbs.

New concerns

Systematic review: bisphosphonate-induced osteonecrosis of the jaws

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Background: Bisphosphonates are used to treat osteoporosis, Paget disease of bone and other metabolic bone diseases, multiple myeloma and skeletal events associated with metastases. In pediatrics, their use is restricted mainly to patients with osteogenesis imperfecta. Their primary mechanism of action is to inhibit osteoclastic bone resorption. Within the past years, many articles have suggested the possible involvement of bisphosphonates in the development of osteonecrosis of the jaws. This review discusses the clinical manifestations, current treatment strategies, possible mechanisms and need for research in the future.

Methods: All published case reports and case series of patients with bisphosphonate-associated osteonecrosis of the jaws were reviewed. Through direct communication with some authors, it was confirmed that some patients were included in multiple reports. When this occurred, data only from the larger, more recent publication was used.

Results/Conclusion: This paper summarizes 368 reported cases of bisphosphonate-associated osteonecrosis of the jaw. Most cases manifested the bone of mandible only (65%), approximately one third of lesions were painless, and there was a slight female predilection among all reported cases. Importantly, 60% of cases occurred after dental surgery procedure. Patients with multiple myeloma and bone metastases who are receiving intravenous, nitrogen-containing bisphosphonates (primarily pamidronate and zoledronic acid) were observed to be at greatest risk for osteonecrosis of the jaws; these patients represented 94% of published cases. The remaining patients were taking oral bisphosphonates for osteoporosis or Paget disease of bone. The most important predisposing factors for the development of bisphosphonate-associated osteonecrosis of the jaws were the type and total dose of bisphosphonate and history of trauma, dental surgery, or dental infection.

Bisphosphonates are drugs that powerfully inhibit bone resorption. They are incorporated into the bone matrix and are thus remarkably persistent drugs. We have had a quick touch on the trigger, but have no indications for how long to treat. It has been noted that prolonged use of bisphosphonates may suppress bone turnover to the point that microdamages cannot be repaired, leading to decreased biomechanical properties. Furthermore, continuing mineralization may yield a hard, brittle bone with an osteopetrotic appearance and an increased risk for fractures. Osteonecrosis of the jaws is a recently described side effect of bisphosphonate therapy, and not a rare one. This review gives an excellent overview based on all reported cases and summarizes current treatment strategies, as well as discusses possible mechanisms of etiology and pathogenesis. Clinical trials are needed to address many issues, e.g. could alternative dosing schedules or monitoring bone turnover markers reduce the incidence? Additional risk factors that may predispose the patient to the development of this condition also need to be studied. The most effective and still safe treatment protocol for these patients still needs to be defined. It should be noted that, e.g., pediatric patients with severe forms of osteogenesis imperfecta often have dental problems, which may require implant surgery. Concurrently, these patients are treated with intravenous pamidronate, which may predispose them for the development of osteonecrosis of the jaws. Thus, constant follow-up of dental health of patients treated with bisphosphonates is required.

Concepts revised or re-centered

Skeletal benefits from calcium supplementation are limited in children with calcium intakes near 800 mg daily

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Osteoporos Int 2006;17:1794–1800

Background: It is still controversial if calcium supplementation improves bone mineral density in children. The authors tested the hypothesis that calcium from milk minerals (MM) but not calcium carbonate (CC) will be beneficial for bone health after discontinuation of supplementation.

Methods: The study included 99 prepubertal boys and girls 5–11 years old who received 800 mg/day of calcium from MM or CC, or a placebo in a 10-month double-blind study. The patients were then followed for 12 months. Total body and regional bone mineral content, and femoral shaft bone dimensions were measured using dual-energy x-ray absorptiometry. Group differences were determined using ANCOVA.

Results: No group differences were observed with regards to increments in bone mineral content or bone dimensions during and after supplementation. Children who remained prepubertal had sustained a greater gain in pelvis bone mineral content in the MM group than controls (37.9 vs. 29.3% respectively, $p < 0.02$).

Conclusion: Calcium supplementation in healthy children already consuming about 800 mg calcium daily does not improve skeletal health. In prepubertal children, calcium supplementation could have a beneficial effect on the pelvis but its biological significance and longevity has to be determined.

Osteoporosis is a major health problem and more than 90% of the peak bone mass is attained by the age of 18 years [7]. Thus, any intervention before that age in order to optimize peak bone mass could have beneficial effects preventing osteoporosis later in life or have a more immediate benefit. A public health intervention could be targeted to diet and/or physical activity. Calcium supplementation has been used in post-menopausal women to improve bone mineral density although only a small positive effect was reported (1.13–2.05%) [8]. Data concerning calcium supplementation in children have not helped us to clarify whether there is a benefit in healthy children already receiving the recommended daily intake, and whether any benefit is sustained after supplementation. The authors conducted a randomized double-blind placebo-controlled trial which shows no benefit of calcium supplementation on bone mineral content except in the pelvis of prepubertal children, a finding of

questionable biological meaning. In support of this work, a recent meta-analysis confirms that calcium supplementation has little effect on skeletal health in healthy children (reviewed in the chapter 'evidence based medicine' of this book) [9]. Therefore, the general belief endorsed by many primary care physicians and mass media that calcium supplementation in children makes bones stronger is not totally right. Our efforts have to be focused on ensuring that all children receive 800 mg calcium per day, and most importantly – vitamin D. Remember, one glass of milk contains 200 mg of calcium. Physical activity is by all means more important than any of the supplements.

Important observations for clinical practice

Reduced bone mineral density and increased bone metabolism rate in young adult patients with 21-hydroxylase deficiency

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Background: Patients with congenital adrenal hyperplasia (CAH) are on long-term glucocorticoid therapy. On the other hand, glucocorticoid therapy is the most frequent cause of drug-induced osteoporosis. This study aimed at evaluating bone mineral density (BMD) and bone metabolism in young adult CAH patients.

Methods: A cross-sectional observational study was conducted at a referral center for pediatric endocrinology. Thirty young patients with the classical form of CAH (aged 16.4–29.7 years) treated with glucocorticoid from diagnosis (duration of treatment 16.4–29.5 years) and 138 healthy controls (aged 16.0–30.0 years) were enrolled. BMD in the lumbar spine and whole body was measured by dual-energy x-ray absorptiometry (DXA). Biochemical markers for bone formation (BALP) and resorption (CTX) were measured in serum.

Results: Height was significantly lower in CAH patients than in controls (women –6.8 and men –13.3 cm). Therefore, several different methods were used to correct for the effect of this difference on BMD measurements. In CAH patients, whole-body BMD measurements were significantly lower than in controls ($p < 0.03$), after correcting for height. No differences were found in lumbar spine BMD. The biochemical markers of bone metabolism were higher in CAH patients than in control subjects ($p < 0.04$). No significant correlations were observed in BMD measurements or bone markers with the actual glucocorticoid dose or mean dose over the previous 7 years.

Conclusion: Young adult patients with the classical form of CAH, receiving continuous glucocorticoid treatment from the very first months of their lives, are shorter and have decreased BMD. Furthermore, their bone metabolism rate is higher. This may put them at risk of developing osteoporosis later in life.

Life-dependent long-term glucocorticoid therapy in CAH patients could potentially cause bone loss and bone fragility. Previous reports of bone mass in CAH patients on glucocorticoid-therapy have showed contradictory results (increased, decreased or normal BMD) [10–12]. Thus, the authors of this paper aimed to measure bone mass and metabolism in young adult patients with the classical form of CAH. Their results demonstrate that CAH patients were significantly shorter than controls. It is well known that DXA measurements are greatly influenced by bone size, and there will be an overestimation of bone deficits in children if the height is not taken into account. Thus, the authors used different methods to account for the height differences. Different approaches yielded that the observed lower total body BMD in CAH patients was real and not size-dependent. Increased levels of biochemical markers for bone metabolism also indicated a high bone turnover state, leading to a low bone mass. However, no correlation was observed between the glucocorticoid dose and BMD/bone markers, suggesting other underlying mechanisms. Premature growth plate fusion, commonly seen in CAH patients, may shorten the time for bone accrual and lead to lower peak bone mass. Therefore, it is possible that the low peak bone mass, rather than glucocorticoid treatment per se, predisposes these patients to osteoporosis later in life. Longitudinal studies are needed to elucidate this issue.

Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells

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Background: Bone remodeling is a tightly regulated process involving the coordinated actions of bone-resorbing osteoclasts and bone-forming osteoblasts. These actions are carried out on the endosteal bone surface, which is close to the microenvironment, or niche, of hematopoietic stem cells (HSCs). The majority of stem cells are located within the bone marrow but a small population is constitutively released to the circulation. However, the mechanisms of this release are unknown. The aim of this study was to investigate the potential role of osteoclasts in homeostasis and stress-induced mobilization of hematopoietic precursors.

Methods: Balb/c, C57BL/6 and protein tyrosine phosphatase ϵ (PTP ϵ) knockout mice were bled or injected with stromal cell-derived factor 1 (SDF-1), hepatocyte growth factor (HGF), lipopolysaccharide (LPS) or receptor activator of NF κ B ligand (RANKL) to induce mobilization and the bones were stained for TRAP which is a phosphatase produced by active osteoclasts. Peripheral blood precursors and white blood cells were counted and analyzed with FACS. Levels of SDF-1 and stromal cell factor (SCF) were measured and osteoclast precursor formation was assayed ex vivo.

Results: Appearance of bone-resorbing osteoclasts along the endosteal surfaces was observed by two different types of physiological stress: mild bleeding and LPS administration mimicking bacterial inflammation. This process was associated with mobilization of hematopoietic progenitors from bone marrow to the circulation. A similar effect was observed by in vivo administration of SDF-1 or HGF. Furthermore, progenitor mobilization was reduced in the presence of calcitonin (known osteoclast inhibitor) but induced with administration of RANKL (known osteoclast stimulator). RANKL administration in vivo was associated with increased expression of MMP-9 and cathepsin K, which in turn reduced the components of the endosteal niche, such as osteopontin and SCF, allowing the release of progenitors. SDF-1 and SCF were demonstrated to be cleaved by cathepsin K in vitro. Interestingly, PTP ϵ -deficient mice, which have a defect in osteoclast adhesion and bone resorption capacity, had lower levels of progenitors and stem cells in their blood. RANKL did not induce progenitor mobilization in these mice.

Conclusion: Osteoclasts have a unique role in the regulation of stress-induced recruitment and release of bone marrow progenitors. Osteoblast-osteoclast balance may be the major regulator of hematopoiesis and turnover of the stem cell niche. Furthermore, RANKL could be considered as a potential agent for clinical transplantation protocols.

A new function for the osteoclast – to mediate stem cells mobilization. HSCs reside in the bone marrow, in specialized microenvironmental domains of the endosteal surfaces, which are called niches. Niches provide HSCs with signals required for a stationary (quiescent) state, during which very few HSCs are released to the circulation. However, the levels of circulating precursors are dramatically increased in response to chemotherapy, irradiation, cytokine stimulation and other stress signals. The authors of this elegant paper demonstrate a new participant in the endosteal niche of HSCs, the bone-resorbing osteoclast. They observed that bleeding or LPS induced stem cell mobilization was associated with increased numbers of osteoclasts on the endosteal surfaces. RANKL, which is a potent osteoclastogenic factor, not only increased osteoclastogenesis (as expected) but also induced stem cell mobilization. This was accompanied by increased expression of osteoclastic proteases, such as MMP-9 and cathepsin K, as well as decreased expression of osteopontin and SCF. The link between osteoclast activation and HSC mobilization was strengthened by experiments with PTP ϵ -deficient mice, which have dysfunctional osteoclasts. These mice had fewer precursors in their bloodstream and bone marrow stem cells were not mobilized in response to RANKL. In summary, it seems that remodeling of bone tissue and remodeling of bone marrow niches are closely coupled. The authors demonstrate that activation of osteoclasts and mobilization of stem cells overlap and depend on the

same signals. It is possible that anti-bone resorptive treatments impair progenitor cell mobilization and thus have clinical implications on bone marrow transplantation procedures. We used to think of the osteoclast-osteoblast unit. The new unit now includes stem cells.

Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts

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Background: $1\alpha,25$ -dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] is crucial for calcium homeostasis and therefore for normal bone metabolism, mainly through actions in the intestine, kidneys and bone. The direct role of the vitamin D receptor (VDR) in bone metabolisms is questionable since calcium supplementation rescues hypocalcemia and hyperparathyroidism and restores bone mineralization both in patients and mice with loss of VDR function. Based on that, using mice with conditional inactivation of the VDR in chondrocytes, the authors studied whether the VDR is involved in growth-plate development and endochondral bone formation.

Results: The lack of VDR in chondrocytes had no effect on growth-plate development but vascular invasion was impaired and osteoclast number was reduced in young mice resulting in increased trabecular bone mass. In vitro experiments revealed that VDR signaling in chondrocytes directly regulate osteoclastogenesis by inducing the expression of receptor activator of NF- κ B ligand (RANKL). Despite that the VDR was specifically inactivated in chondrocytes, mineral homeostasis in young mice, in whom growth-plate activity is important, was also affected since serum phosphate and $1,25(OH)_2D_3$ levels were increased. Further experiments clarified the mechanism: VDR inactivation in chondrocytes decreased the expression of FGF23 by osteoblasts and subsequently increased renal expression of 1α -hydroxylase and of the sodium phosphate cotransporter type IIa.

Conclusion: This study shows that VDR signaling in chondrocytes is not essential for growth-plate development but it is important for osteoclast formation during bone development and for the endocrine action of bone in phosphate homeostasis.

$1,25(OH)_2D_3$ is one of the major regulators of calcium homeostasis mainly through actions in intestine and kidneys, whereas its actions on bone are controversial. Despite in vitro data in the literature, indicating that VDR has actions on bone, the controversy has risen from patients and mice with loss of VDR function. Calcium supplementation has been shown to rescue hypocalcemia and hyperparathyroidism and to restore bone mineralization in both patients and mice [13, 14]. In addition, mice lacking VDR had growth-plate abnormalities before the onset of hypocalcemia, suggesting a role of VDR signaling in endochondral bone formation. Several findings in this article are surprising and very interesting. A new role of the growth plate was revealed; the growth plate as a co-player in the endocrine regulation of bone. Thus, the growth-plate chondrocytes, especially in young rapidly growing mice, contribute to the endocrine negative feedback loop between $1,25(OH)_2D_3$ and FGF23. Moreover, the findings support the existence of a soluble chondrocyte-derived factor inducible by $1,25(OH)_2D_3$ which stimulates FGF23 production by osteoblasts. Further research is needed to confirm the paracrine role of the growth plate in the regulation of bone metabolism and also to define the underlying mechanisms.

FGF18 is required for early chondrocyte proliferation, hypertrophy and vascular invasion of the growth plate

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Background: Fibroblast growth factor-18 (FGF18) has been shown to regulate chondrocyte proliferation and differentiation by signaling through FGF receptor-3 (FGFR3) and to regulate osteogenesis by signaling through other FGFRs. Fgf18^{-/-} mice have an apparent delay in skeletal mineralization that is

not seen in *Fgfr3*^{-/-} mice and thus, this delay in mineralization cannot be simply explained by FGF18 signaling to osteoblasts.

Methods: Skeletal explants of *Fgf18*^{-/-} mice were studied. Bone mineralization, growth plate morphology and expression patterns were studied by immunohistochemistry and in situ hybridization.

Results: A delayed mineralization in *Fgf18*^{-/-} mice was shown to be closely associated with delayed initiation of chondrocyte hypertrophy, decreased proliferation at early stages of chondrogenesis, delayed skeletal vascularization and delayed osteoclast and osteoblast recruitment to the growth plate. FGF18 was found to be necessary for the expression of vascular endothelial growth factor (VEGF) in hypertrophic chondrocytes and perichondrium, and be sufficient to induce VEGF expression in skeletal explants.

Conclusion: The findings support a model in which FGF18 regulates skeletal vascularization and subsequent recruitment of osteoblasts/osteoclasts through regulation of early stages of chondrogenesis and VEGF expression. FGF18 thus coordinates neovascularization of the growth plate with chondrocyte and osteoblast growth and differentiation.

We barely adopted FGF23, and here comes another family member. The authors demonstrate delayed ossification in *Fgf18*^{-/-} mice which is closely associated with delayed initiation of chondrocyte hypertrophy, skeletal vascularization and osteoclast recruitment, and decreased chondrocyte proliferation. The findings support a model in which FGF18 regulates skeletal vascularization and osteoclast recruitment through regulation of VEGF signaling. The data presented also support a role for FGF18 to regulate chondrocyte proliferation and differentiation by signaling via FGFR3 (gain of function in achondroplasia) in proliferating chondrocytes and osteogenesis by signaling via FGFR1 and/or FGFR2 in the perichondrium/periosteum. It can be concluded that FGF18 functions in coordinating the critical developmental stages of endochondral ossification by balancing the developmental timing of chondrogenesis, osteogenesis and vascularization. The functional consequences of disturbed FGF18 function in humans remains to be described. If you have a child with delayed bone mineralization and poor growth, you might have a case of mutated FGF18!

New genes

Deficiency of cartilage-associated protein in recessive lethal osteogenesis imperfecta

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Background: The classic form of osteogenesis imperfecta (OI) is caused by mutations in the genes for type I collagen. A recessive form of OI has long been suspected in some cases, where unaffected parents have more than one child with severe bone dysplasia. Since loss of cartilage-associated protein (CRTAP) causes severe osteoporosis in mice, the possible role of the CRTAP gene in lethal or severe OI was studied.

Methods: Dermal fibroblasts from 10 children with lethal (type II) or very severe (type III) OI were sampled and CRTAP mRNA levels were determined by RT-PCR. These children had normal primary structure of type I collagen with excess post-translational modifications in the helical region of the α -chain. Several different controls were also examined. Three children with decreased CRTAP mRNA levels were screened for 7 CRTAP exons and surrounding intronic sequences. Western blot of CRTAP protein and analysis of collagen modifications were performed.

Results: In 3 infants (1, 2 and 3) CRTAP mRNA levels were 0–25% of the normal value. The parents of infants 1 and 3 had normal levels of CRTAP mRNA, indicating that there was more than one defective allele involved. The exons and surrounding introns of infants 1, 2 and 3 were sequenced, and mutations were identified. Mutations led to premature termination of translation, triggering intracellular destruction of the mutant mRNA transcripts. At the protein level, no intracellular CRTAP was detectable in fibroblasts from infants 1, 2 and 3, and prolyl 3-hydroxylation of type I collagen was minimal.

Conclusion: Three of 10 children with lethal or severe OI without primary collagen defects were found to have a recessive condition resulting in CRTAP deficiency. CRTAP deficiency led to no or minimal Pro986 hydroxylation near the C-terminus of type I collagen, and the spectrum of mutations suggests that prolyl 3-hydroxylation is crucial for normal bone formation. It is possible that this process is important for other organs as well, contributing to the lethal outcome.

The clinical picture of OI patients is heterogeneous with symptoms extending from mild to perinatal lethality. Most cases are caused by mutations in type I collagen gene, and the pathogenesis is related to quantitative and qualitative abnormality of collagen. OI is usually autosomal dominant, and a recessive form has long been suspected but candidate genes have yet not been identified. Morello et al. demonstrated that loss of CRTAP in mice causes an osteochondrodysplasia which is characterized by severe osteoporosis, decreased osteoid production and decreased prolyl 3-hydroxylation of collagens in bone and cartilage [15]. Furthermore, Morello et al. [15] demonstrated that in humans, CRTAP mutations were associated with a clinical spectrum of recessive OI. Complete loss of CRTAP led to more severe forms of OI. These findings were partially confirmed by Barnes et al., who screened skin samples from 10 children with lethal or severe OI with normal primary structure of type I collagen. They were able to identify null mutations in the CRTAP gene leading to a lethal condition with death occurring within the first year of life. In summary, variable loss of CRTAP function and prolyl 3-hydroxylation seem to be a mechanism for recessive OI and may contribute to new cases, whose previous diagnosis has only been based on alterations in type I collagen. Indeed, the involvement of the prolyl 3-hydroxylase gene (LEPRE1) in a recessive metabolic bone disorder resembling lethal or severe OI has recently been published [16]. The authors present 5 cases resulting from LEPRE1 null alleles, whose phenotypes overlap with severe OI but also have distinctive features, such as lack of blue sclerae, a round face and a short chest. Taken together, these findings suggest that the classification and genetical diagnosis of OI and related disorders is far from complete.

Functional characterization of heterogeneous nuclear ribonuclear protein C1/C2 in vitamin D resistance

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Background: Almost all of the cases with hereditary vitamin D-resistant rickets (HVDRR) are caused by homozygous mutations (loss of function) in the vitamin D receptor (VDR). In 2003, the authors described a patient with the classical HVDRR phenotype but normal VDR function [17]. This patient was hormone-resistant because of a constitutive overexpression of the heterogeneous nuclear ribonucleoprotein (hnRNP) which competed with a normally functioning VDR-retinoid X receptor dimer for binding to the vitamin D response element (VDRE). In the present study the authors purified, cloned, and further clarified the function of this competitive response element-binding protein (REBiP) hnRNP C1/C2 causing vitamin D resistance.

Results: The authors purified and cloned the overexpressed VDRE from their HVDRR patient (with no VDR mutation). It was a competitive response element-binding protein (REBiP) hnRNP C1/C2. This gene produces two alternatively spliced translated products (hnRNP C1 and C2). Then, cDNAs for hnRNP C1 and hnRNP C2 inhibited VDR-VDRE-directed transactivation when they were overexpressed in vitamin D-responsive cells. In addition, overexpression of hnRNP C1/C2 exerted a dominant-negative effect on 1,25(OH)₂D₃-driven VDRE promoter activity. In the presence of 1,25-dihydroxyvitamin D, a cyclical movement of VDR and VDRE starts association-dissociation. The authors found that these receptor response element cycling events happened due to the competitive presence of the REBiP at the VDRE. The temporal and reciprocal pattern of VDR and hnRNP C1/C2 interaction with the VDRE was lost in HVDRR cells overexpressing the hnRNP C1/C2 REBiP.

Conclusion: The data indicate that REBiP is a component of the multiprotein complex involved in the regulation of vitamin D-mediated transcription and most likely REBiP is guiding the cyclical on-off equilibrium between VDR and VDRE.

Most cases of steroid hormone resistance are caused by end-organ resistance due to inactivating mutations of genes encoding their receptors. At least one case has been reported with a mutation not in the receptor but in one of those accessory proteins [18]. A patient with androgen insensitivity syndrome had an intact receptor but an abnormal coactivator protein which interacts with the AF-1 domain of the androgen receptor [18]. The authors of the present article have described another patient with the classical phenotype of HVDRR but normal VDR. In their previous attempt to find out the mechanism they discovered that this patient overexpressed hnRNPs which competed with a normally functioning VDR-retinoid X receptor dimer for binding to the VDRE. Now the authors went one step further. They cloned this protein and elucidated the mechanism of action. This protein is hnRNP C1/C2, a ribonucleoprotein which also functions as a competitive response element-binding protein. $1,25(\text{OH})_2\text{D}_3$ initiates a cyclical movement of the VDR being associated and dissociated from the VDRE. hnRNP C1/C2 competes with the VDR for VDRE occupancy and therefore overexpression of hnRNP C1/C2 prevents the VDR to bind to the VDRE. Since VDR-VDRE association is necessary for VDR signaling, the result is resistance to VDR. These findings could also apply to other steroid and nuclear receptors since coactivators, corepressors and response element binding proteins play a key role in their proper function. As has been mentioned, there are patients with androgen resistance with no mutations in the androgen receptor, patients with resistance to multiple steroid hormones, or with a thyroid hormone resistance phenotype but no mutations in the thyroid hormone receptors [19]. These patients could have mutations of elements important for the proper function of their respective receptors.

New hormones/Reviews

C-type natriuretic peptide in growth: a new paradigm

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Background: C-type natriuretic peptide (CNP), acting through its receptor, natriuretic peptide receptor-B (NPR-B), plays a critical role in linear growth. Knockout mice for CNP and NPR-B are dwarfed, and transgenic mice overexpressing CNP are overgrown. CNP has a direct regulatory effect on growth plate chondrocytes, acting primarily to promote terminal differentiation and hypertrophy.

Results: In humans, homozygous NPR-B mutations are the cause of acromesomelic dysplasia, Maroteaux type (AMDM). A patient with AMDM and the NPR-B knockout mouse both have low IGF-1 levels, suggesting an interaction between these regulatory systems. Heterozygous carriers of NPR-B mutations also have reduced stature, but no other abnormalities. Hence, heterozygous NPR-B mutations are another cause of 'idiopathic' short stature.

Conclusion: The CNP–NPR-B system has only recently been found to be an important regulator of human growth, and abnormalities in this system have clinical implications. Considerable work is needed to further understand this new paradigm of human growth regulation.

Data from mouse models and in vitro studies have shown that CNP, acting through its receptor NPR-B, plays a critical role in the regulation of the growth plate and hence in linear growth. The recent discovery of NPR-B mutations as a cause for acromesomelic dysplasia, Maroteaux type (AMDM), a severe form of dwarfism in humans, demonstrates that the CNP–NPR-B regulatory system is also critical to human linear growth. Preliminary findings suggest that the CNP–NPR-B system also interacts with the GH-IGF-1 system, which may have an additional, indirect role in linear growth. As discussed in this review, heterozygous carriers of inactivating NPR-B mutations have reduced stature. These carriers lack the skeletal disproportion and abnormal IGF-1 levels of AMDM and would clinically be diagnosed with 'idiopathic' short stature. Since the prevalence of AMDM in the general population is 1 in 2,000,000, the prevalence of heterozygous NPR-B mutation carriers is 1 in 700 (assuming Hardy-Weinberg equilibrium). The authors present data showing that these carriers have a mean height

z-score of -1.8 . Using the definition of a height z-score of less than -2.25 for 'idiopathic' short stature, about 1 in 30 of these individuals would be carriers of NPR-B mutations. Based on the information given, it can be assumed that heterozygous NPR-B mutations may be a significant cause of 'idiopathic' short stature. Further studies are needed to confirm this and also whether these patients do respond better to GH than other patients with 'idiopathic' short stature.

Food for thought

Regulation of fibroblast growth factor-23 signaling by Klotho

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Background: Klotho is a gene suppressing aging and Klotho knockout mice have a variety of aging-like phenotypes, many of them similar to those observed in fibroblast growth factor-23 (FGF23) knockout mice. The authors aimed to study whether Klotho and FGF23 use common signal transduction pathway(s).

Results: Experiments revealed that the Klotho protein directly binds to multiple FGF receptors (FGFRs) and then the Klotho-FGFR complex binds to FGF23 with higher affinity than FGFR or Klotho alone. Furthermore, Klotho increased the ability of FGF23 to induce phosphorylation of FGF receptor substrate and ERK.

Conclusion: The data indicate that Klotho can act as a cofactor important for the activation of FGF signaling by FGF23.

Hypervitaminosis D and premature aging: lessons learned from FGF23 and Klotho mutant mice

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Background: Mice lacking Klotho (K1) or fibroblast growth factor-23 (FGF23) have similar phenotypes concerning aging. In addition, both have increased levels of vitamin D and altered mineralization. On the other hand, it is well known that aging is associated with decreased levels of vitamin D. Based on this knowledge, the authors hypothesized that high levels of vitamin D could limit the aging process.

Results: The article reviews data from human diseases caused by hyper- and hypofunction of FGF23 and genetically modified mice. FGF23 knockout mice have premature aging similarly to Klotho-deficient mice. Both mice have hypercalcemia and increased serum levels of $1,25(\text{OH})_2\text{D}_3$. When the activity of vitamin D was eliminated or reduced in K1 and FGF23 knockout mice, the aging process was decelerated with prolonged survival.

Conclusion: Based on the above data in the literature, the authors conclude that increased vitamin D activity could accelerate aging.

The new hormone Klotho was discussed for the first time in our 2006 Yearbook [20]. Both Klotho and FGF23 are related to aging in mice. Experiments with transgenic mice have revealed their involvement in accelerated aging [21]. Most surprisingly, FGF23 and Klotho-deficient mice have similar aging phenotypes indicating that these two proteins may function through a common signaling pathway. Indeed, the article by Kurosu et al. clearly shows that Klotho is a cofactor important for the activation of FGF signaling by FGF23. Klotho binds to FGFR and this complex binds to FGF23 with higher affinity than FGFR or Klotho alone. FGF23 is a phosphaturic protein and it is involved in human diseases of

phosphate homeostasis. In addition to phosphorus, FGF23 negatively regulates vitamin D in mice and humans and Klotho in mice. In addition to premature aging, both Klotho and FGF23-deficient mice have increased vitamin D due to increased renal expression of 1 α -hydroxylase. The second article by Razzaque and Lanske raises a very interesting question based on data in literature: Is hypervitaminosis D associated with accelerated aging? The question is very important with potential impact on public health since many postmenopausal women are treated with high doses of vitamin D. The authors raised this question based on studies in transgenic FGF23, Klotho, and 1 α -hydroxylase mice. They noticed that the common link in FGF23 and Klotho-deficient mice is premature aging with increased serum levels of vitamin D. This phenotype was reversed when the 1 α -hydroxylase gene was knocked out in addition to FGF23 or when Klotho-deficient mice were on vitamin D-deficient diet. This research area is now very exciting revealing an unexpected role of vitamin D. For sure, we will soon learn more about the role of vitamin D in the regulation of aging.

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