Bone, Growth Plate and Mineral Metabolism

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It is a challenge to select only a handful of papers from the abundance of high-quality new literature on skeletal physiology and pathology. Last year's chapter described the expanding role of the skeleton in whole-body homeostasis [1]; the present knowledge has been thoroughly covered in a review article by Karsenty and Feron [2]. This year's selected papers discuss new mechanisms in osteoblast and osteoclast communication and their functional regulation. New therapies, such as enzyme-replacement therapy for life-threatening hypophosphatasia and a novel tyrosine kinase inhibitor for achondroplasia, although in their early phases, provide new hope for these debilitating illnesses. The whole-exome sequencing approach has uncovered the genetic background of 'idiopathic' infantile hypercalcemia and acrodysostosis. These discoveries open exciting avenues for further research and are likely to expand our knowledge on not only the pathology behind the disorders but also on the physiological processes in mineral and skeletal homeostasis. Such development can be seen in hereditary vitamin D-resistant rickets caused by unfunctioning vitamin D receptor. The lively discussion on vitamin D target levels – still poorly defined for the pediatric age group – continues. But in the midst of these debates it is good to be reminded about the importance of accurate means to measure serum 25-OH-vitamin D: if the results are unreliable, the target levels are of little value.

Mechanism of the year Acrodysostosis is Albright's close relative

Recurrent *PRKAR1A* mutation in acrodysostosis with hormone resistance

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N Engl J Med 2011;364:2218–2226

Background: The skeletal features of acrodysostosis resemble the Albright's hereditary osteodystrophy in patients with pseudohypoparathyroidism type 1a, but defects in the α -stimulatory subunit of the G-protein (GNAS) are not present in patients with acrodysostosis.

Methods: The authors used candidate-gene approach to test the hypothesis that a gene defect elsewhere in the cyclic AMP (cAMP)-dependent protein kinase A signaling pathway downstream of GNAS might be involved.

Results: Three unrelated patients with acrodysostosis and resistance to multiple hormones were studied. The authors found a heterozygous germline mutation p.R368X in the gene encoding *PRKAR1A*, the cAMP-dependent regulatory subunit of protein kinase A in all 3 patients. Functional studies showed that the mutated subunit impaired the protein kinase A response to stimulation by cAMP.

Conclusion: Acrodysostosis with hormone resistance is caused by *PRKAR1A* mutation and decreased protein kinase A sensitivity to cAMP. This explains hormone resistance and skeletal similarities with patients with pseudohypoparathyroidism type 1a.

Exome sequencing identifies *PDE4D* mutations as another cause of acrodysostosis

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Am J Hum Genet 2012;90:740–745

Background: Acrodysostosis is a rare autosomal-dominant condition with facial dysostosis, severe brachydactyly, and short stature. Moderate intellectual disability may also be present. Recently, a recurrent mutation in *PRKAR1A* has been identified in 3 individuals with acrodysostosis and resistance to multiple hormones.

Methods: The authors looked for *PRKAR1* mutations in 10 unrelated patients with acrodysostosis, and performed exome sequencing in mutation-negative patients.

Results: De novo *PRKAR1A* mutations were identified in 5 out of the 10 individuals. Four patients had p.Arg368* and 1 had p.Tyr373His. Exome sequencing identified heterozygous mutations in the phosphodiesterase 4D (*PDE4D*) gene as another cause for acrodysostosis. *PDE4D* encodes a class IV cAMP-specific phosphodiesterase that regulates cAMP concentration. Altogether, 4 out of 10 patients harbored heterozygous de novo *PDE4D* mutations (p.Pro225Thr, p.Phe226Ser, p.Ser190Ala and p.Thr587Pro). The 4 individuals with *PDE4D* mutations shared common clinical features, namely characteristic mid-face and nasal hypoplasia and moderate intellectual disability, but no hormone resistance. However, resistance to PTH and TSH was consistently observed in the 5 cases with *PRKAR1A* mutations.

Conclusion: The findings further support the key role of the cAMP signaling pathway in skeletogenesis.

Exome sequencing identifies *PDE4D* mutations in acrodysostosis

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Background: Acrodysostosis is a dominantly-inherited, multisystem disorder with skeletal, endocrine, and neurological abnormalities.

Methods: The authors performed exome sequencing on 5 genetically independent cases with acrodysostosis to identify the underlying genetic cause.

Results: Three different heterozygous missense mutations in *PDE4D*, encoding cAMP-specific phosphodiesterase 4D, were found in 3 of the cases. Two additional cases were heterozygous for de novo missense mutations in *PRKAR1A*, which encodes the cAMP-dependent regulatory subunit of protein kinase A.

Conclusion: These findings demonstrate that acrodysostosis is genetically heterogeneous and underscore the exquisite sensitivity of many tissues to alterations in cAMP homeostasis.

These three papers elucidate the genetic defects underlying acrodysostosis, a rare autosomal dominant skeletal dysplasia. The condition has some similarity with pseudohypoparathyroidism 1A, especially when hormone resistance is present. Several hormones activate G-protein-coupled receptors, which then activate G-protein and adenylyl cyclase, generating intracellular cAMP. In turn, cAMP activates protein kinase A, resulting in the phosphorylation of specific proteins that mediate the effects of these hormones. In pseudohypoparathyroidism type 1a defective α -stimulatory subunit of the G-protein (GNAS) fails to activate the signaling cascade. These three papers show that defects within the same signaling pathway but downstream of GNAS underlie acrodysostosis (fig. 1). The main clinical features of acrodysostosis include facial dysostosis with nasal hypoplasia, severe brachydactyly, and short stature. Moderate intellectual disability may be present. Some patients have resistance to multiple hormones, including PTH, TSH, growth hormone-releasing hormone, and gonadotropins. Altogether, four different *PRKAR1A* mutations were identified in 10 of the reported 18 patients with acrodysostosis; one of the mutations was recurrent and all mutations were located in exon 11. As in other skeletal dysplasias, these are gain-of-function mutations as they decrease protein kinase A sensitivity to cAMP. Seven patients were found to have different heterozygous de novo mutations in the *PDE4D* gene. Phenotype-genotype correlation suggested that patients with *PRKAR1A* mutations were

Fig. 1. Ligand binding activates Gs- α and stimulates cAMP synthesis. PRKAR1A binds cAMP, leading to dissociation and activation of PKA and the subsequent phosphorylation of cAMP response element binding (CREB), nuclear translocation, and expression of target genes. PDE4D activity modulates cAMP levels. The asterisks indicate mutated genes in Albright hereditary osteodystrophy (*GNAS*) and in acrodysostosis (*PDE4D* or *PRKAR1A*).

more likely to have short stature and hormone resistance while patients with *PDE4D* gene mutations had the characteristic facial dysostosis and intellectual disability, usually normal stature and no hormone resistance. There was however phenotypic overlap, which is not surprising considering that the defects involve the same signaling cascade. These three reports provide intriguing new information regarding the cAMP-mediated signaling in disorders within pediatric endocrinology.

New mechanisms All the good in one molecule?

Osteoprotection by semaphorin 3A

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Background: Skeletal homeostasis requires in addition to hormones also local factors that regulate bone-forming osteoblasts and bone-resorbing osteoclasts. Osteoprotegerin protects bone by inhibiting osteoclastic bone resorption, but no factor has yet been identified that could locally regulate both osteoclasts and osteoblasts.

Methods: The authors used osteoblasts from mice that lacked osteoprotegerin to identify other factors involved in the regulation of bone homeostasis locally. The function of the identified semaphorin 3A (Sema3A) was then characterized in various animal models and cell assays.

Results: Sema3A was found to exert an osteoprotective effect by both suppressing osteoclastic bone resorption and increasing osteoblastic bone formation. The binding of Sema3A to neuropilin-1 (Nrp1) inhibited RANKL-induced osteoclast differentiation. In addition, Sema3A and Nrp1 binding stimulated osteoblast and inhibited adipocyte differentiation through the canonical Wnt/ β -catenin signaling

pathway. The *Sema3a*^{-/-} mice displayed osteopenic phenotype; a similar phenotype was shown in mice with a disrupted Sema3A-binding site in Nrp1. Intravenous Sema3A administration in mice increased bone volume and expedited bone regeneration.

Conclusion: The authors conclude that Sema3A inhibits osteoclast differentiation and promotes osteoblastic bone formation and is thus a promising new therapeutic agent in bone and joint diseases.

Bone formation is linked to bone resorption through coupling factors, and skeletal homeostasis requires a balance between these two processes. The differentiation of bone-resorbing osteoclasts is regulated by cells expressing the key osteoclast differentiation factor, RANKL. Osteoblastic cells counterbalance the function of RANKL by producing a soluble decoy receptor for RANKL, osteoprotegerin (Opg). The authors used osteoblast cultures from Opg-deficient mice and show that another factor, Sema3A – a signaling protein involved in the regulation of axonal growth, is a potent direct inhibitor of osteoclastogenesis. Sema3A inhibits the differentiation of osteoclast precursor cells into osteoclasts, thus preventing excessive bone degradation. However, it also promotes osteoblastic bone formation: newly formed osteoblasts secreted Sema3A to augment and coordinate their own differentiation and bone-forming activity. The activity of Sema3A is initiated by its binding to a cell-surface receptor neuropilin-1. Mice deficient of Sema3A and mice with a defective neuropilin-1 receptor, unable to bind Sema3A, both show an osteoporotic phenotype, which is due to enhanced bone degradation and reduced bone formation. The authors went on to investigate whether an intravenous injection of Sema3A could prevent bone loss in a mouse model of menopause. Remarkably, the Sema3A injection inhibited bone degradation and promoted bone formation. Such a potential therapeutic agent would be superior to any presently available medication for the treatment of osteoporosis. The authors optimistically state that Sema3A represents the long sought soluble molecule with the capacity to bring both osteoblasts and osteoclasts into a condition that favors bone mineral increase. The findings are indeed promising and will hopefully lead to the development of a combined antiresorptive and bone-increasing agent capable of promoting bone regeneration.

New mechanisms Moving from D to E in skeletal alphabets

Vitamin E decreases bone mass by stimulating osteoclast fusion

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Background: Bone homeostasis requires a balance between osteoblastic bone formation and osteoclastic bone resorption. Osteoclasts are multinucleated cells that are formed by mononuclear preosteoclast fusion. It is known that vitamin D plays a pivotal role in maintaining skeletal integrity. However, the role of vitamin E in bone remodeling is unknown.

Methods: The authors used mice deficient in α -tocopherol transfer protein (Ttpa^{-/-} mice), a mouse model of genetic vitamin E deficiency, as well as cell-based assays to study the role of vitamin E in bone metabolism.

Results: The Ttpa^{-/-} mice had high bone mass as a result of a decrease in bone resorption. Cell-based assays indicated that α -tocopherol stimulated osteoclast fusion, independent of its antioxidant capacity. This was mediated by induction of the expression of dendritic-cell-specific transmembrane protein (DC-STAMP), which is an essential molecule for osteoclast fusion and encoded by *Tm7sf4*. Indeed, the bone abnormality seen in Ttpa^{-/-} mice was rescued by a *Tm7sf4* transgene. Moreover, wild-type mice or rats fed an α -tocopherol-supplemented diet, which contains a comparable amount of α -tocopherol to common human supplements, lost bone mass.

Conclusion: These results show that serum vitamin E is a determinant of bone mass by regulating osteoclast fusion.

With all the noise about vitamin D and bone, vitamin E seems to have been forgotten; when first identified, its deficiency was believed to cause rickets. In this study the authors apply mouse models to elucidate the mechanisms through which vitamin E might influence skeletal homeostasis. Vitamin E, a lipid-soluble antioxidant, is believed to be protective against arteriosclerotic change and the aging process and is one of the most widely used supplements. Vitamin E is a mixture of tocopherols and tocotrienols. After intestinal absorption it is transported to the liver, where α -tocopherol transfer protein (α -TTP) selectively transfers α -tocopherol into lipoproteins. Consequently, α -tocopherol is the predominant isoform of vitamin E in the body. Mice deficient of α -TTP (*Ttpa*^{-/-} mice) had, compared to wild-type mice, higher bone mass in both vertebrae and long bones as a consequence of lower bone resorption and unaltered bone formation. The skeletal phenotype was reversed by supplemental α -tocopherol. α -Tocopherol stimulated osteoclast differentiation while osteoblast differentiation remained unaltered. Vitamin E affected the later stage of osteoclast differentiation and stimulated osteoclast fusion, resulting in increased osteoclast size, nuclear number and bone-resorbing capacity per osteoclast. Finally, the authors fed the mice with α -tocopherol with doses comparable to those present in vitamin E supplements. This induced a 20% decrease in bone mass with concomitant increase in bone resorption and osteoclast size after 8 weeks, suggesting that excessive use of vitamin E may be deleterious for bone. Human studies on vitamin E and bone health have been limited and show conflicting results. A recent paper by Hamidi et al. [3] evaluated the effects of vitamin E on bone turnover markers among US postmenopausal women. The vitamin E users had significantly higher levels of α -tocopherol while levels of δ -tocopherol, another vitamin E isomer mostly obtained from dietary sources, were lower. This constellation associated with lower levels of bone formation and unaltered bone resorption marker status, suggesting uncoupling of bone formation and resorption. Based on these studies it seems that while E may have beneficial anti-inflammatory and antioxidant properties (poorly documented), it may also mediate adverse skeletal effects with increased bone loss and/or diminished bone formation.

New mechanisms TSH, the osteoblast-stimulating hormone

Thyroid-stimulating hormone induces a Wnt-dependent, feed-forward loop for osteoblastogenesis in embryonic stem cell cultures

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Background: The thyroid-stimulating hormone (TSH) can bypass the thyroid to exert a direct protective effect on the skeleton. Thus in hyperthyroidism a low TSH level, in addition to high thyroid hormone level, may contribute to bone loss. Mouse genetic, cell-based, and clinical studies have previously established that TSH inhibits osteoclastic bone resorption. However, the direct influence of TSH on the osteoblast has remained unclear.

Methods: The authors used a model system developed from murine embryonic stem cells, induced to form mature mineralizing osteoblasts, and measured TSH-induced changes in osteoblastic markers.

Results: TSH stimulated osteoblast differentiation primarily through the activation of protein kinase C δ and the upregulation of the noncanonical Wnt components frizzled and Wnt5a. Their findings suggested that a TSH-induced, fast-forward short loop in bone marrow permits Wnt5a production, which enhances osteoblast differentiation and also stimulates osteoprotegerin secretion to attenuate bone resorption by neighboring osteoclasts.

Conclusion: The authors conclude that TSH promotes osteoblast differentiation and that this osteoblastogenic effect of TSH is mediated by the noncanonical Wnt pathway and that protein kinase C δ is a downstream mediator of TSH action. This loop uncouples bone formation from bone resorption with a net increase in bone mass.

Previous studies have established that TSH receptors are expressed in bone cells and that absent TSH receptor signaling causes high-turnover bone loss [4, 5]. TSH inhibits bone resorption directly by acting on osteoclastic TSH receptors. However, TSH also seems to stimulate bone formation through a direct action on the osteoblast. The authors used embryonic stem cells to further characterize the effects of TSH on osteoblasts. Osteoblastic differentiation of the embryonic stem cells was significantly enhanced in the presence of TSH and activation of the TSH receptor. TSH also potently stimulated the production of osteoprotegerin, an inhibitor of osteoclastic bone resorption. These actions of TSH were primarily exerted through the noncanonical Wnt pathway. Based on these observations, the authors hypothesize that not only low thyroid hormone levels but also high TSH in children with congenital hypothyroidism might have an effect on skeletal development. It remains to be elucidated in future studies whether these observations in embryonic stem cells can be translated to clinical practice.

New paradigms The multi-tasking osteocyte

Demonstration of osteocytic perilacunar/canalicular remodeling in mice during lactation

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Background: Lactation is associated with a significant decline in bone mineral density (BMD) both in humans and mice. Osteoclasts are thought to be solely responsible for the removal of bone matrix. The authors hypothesized that osteocytes might also play a role in mobilizing bone mineral during lactation.

Methods: Various mouse models were used to study the effects of lactation, weaning and unloading on gene expression profiles, BMD, bone microstructure and scanning electron microscopy findings.

Results: Gene array analysis of osteocytes from lactating animals revealed an elevation of genes known to associate with osteoclastic bone resorption including tartrate-resistant acid phosphatase (TRAP) and cathepsin K that returned to virgin levels upon weaning. Infusion of PTHrP, known to be elevated during lactation, induced TRAP activity and cathepsin K expression in osteocytes concurrent with osteocytic remodeling. Conversely, animals lacking the PTH type 1 receptor in osteocytes failed to express TRAP or cathepsin K or to remodel their osteocyte perilacunar matrix during lactation.

Conclusion: The findings show that osteocytes remove mineralized matrix through molecular mechanisms similar to those utilized by osteoclasts. Osteocytes thus have an active role in the regulation of calcium homeostasis during lactation.

In humans, lactation associates with a 5–8% decline in maternal BMD and may occasionally lead to severe symptomatic postpartum osteoporosis with vertebral fractures [6]. Previous studies have suggested that this is not only caused by increased osteoclast activity but other mechanisms may play a role. The authors here elegantly show that osteocytes can remove mineral from their surroundings and subsequently replace it in a cyclical fashion. This so-called osteocytic osteolysis seems to be a part of normal adaptation of bone and calcium metabolism to the demands of reproduction. This study provides more evidence for the significant role of the osteocyte in bone health and disease. Osteocytes are the most abundant cells in the bone tissue. They orchestrate skeletal remodeling by regulating osteoblasts and osteoclasts on the bone surface, participate in phosphate homeostasis, and mediate the effects of mechanical forces on the skeleton. And as shown here, they also participate in calcium homeostasis. A truly good all-around player in the bone!

miR-34s inhibit osteoblast proliferation and differentiation in the mouse by targeting SATB2

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Background: It has been suggested that microRNAs (miRNAs) may be involved in the regulation of osteoblast proliferation and/or differentiation but this has not been shown in vivo.

Methods: The authors used osteoblast cell lines to screen for microRNAs whose expression increases during osteoblast differentiation. The function of the miRNAs was studied in various osteoblast-specific gain- and loss-of-function mouse models.

Results: The authors identified members of the miR-34 family as regulators of osteoblast proliferation and differentiation. Further in-vivo experiments revealed that miR-34b and -34c affected skeletogenesis during embryonic development, as well as bone mass accrual after birth, through two complementary cellular and molecular mechanisms. First, they inhibited osteoblast proliferation by suppressing cyclin D1, CDK4, and CDK6 accumulation. Second, they inhibited terminal differentiation of osteoblasts, at least in part through the inhibition of SATB2, a nuclear matrix protein that is a critical determinant of osteoblast differentiation. Genetic evidence obtained in the mouse confirmed the importance of SATB2 regulation by miR-34b/c.

Conclusion: These results are the first to identify a family of microRNAs involved in bone formation in vivo and to identify a specific genetic pathway by which these microRNAs regulate osteoblast differentiation.

MicroRNAs (miRNAs) are small noncoding RNAs that downregulate expression of their target genes by either mRNA degradation or translational inhibition. They thus provide a novel dimension to post-transcriptional control by directly inhibiting mRNA translation. This mode of action implies that regulation of mRNA and/or protein levels can proceed faster than transcriptional mechanisms. Consequently, miRNAs mediate rapid fine tuning of gene expression. Previous studies have suggested that miRNAs are involved in osteoblast maturation [7]. However, in-vivo data have been lacking. In this study the authors screened for miRNAs whose expression significantly increased during osteoblast differentiation. Of the several potential miRNAs, they selected miR-34b and miR-34c for further studies since, in addition to increased expression during osteoblastogenesis, their expression was restricted mainly to osteoblasts. Mice overexpressing miR-34c in the osteoblasts had low bone mass with decreased osteoblast number and bone formation rate, while osteoclast activity was unchanged. In contrast, osteoblast-specific ablation of miR-34b and -34c resulted in significant increase in bone mass and osteoblast number. The authors identified specific targets for miR-34-mediated regulation. One of these was SATB2, which is a transcription factor belonging to the family of special AT-rich binding proteins and involved in craniofacial patterning and osteoblast differentiation [8]. This work shows that miRNAs have an important role in the regulation of bone formation. In fact, it is likely that miRNAs contribute to every phase of skeletogenesis, from embryonic bone development to maintenance of adult bone homeostasis by regulating the growth, differentiation and activity of the involved cells; the current knowledge has been elegantly reviewed by Lian et al. [9]. However, the role of miRNAs in pathologic human skeletal disorders such as osteoporosis and osteoarthritis remains largely unknown.

Denosumab treatment for fibrous dysplasia

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J Bone Miner Res 2012;27:1462–1470

Background: Fibrous dysplasia (FD) is caused by somatic activating mutations in the cAMP-regulating protein, Gs- α . These lead to replacement of normal bone by proliferative osteogenic precursors, resulting in bone deformity, fractures, and pain. RANKL, a cell surface protein involved in osteoclastogenesis, is overexpressed in FD. This prompted the authors to use denosumab, a humanized monoclonal antibody to RANKL, in the treatment of FD.

Methods: The authors present a 9-year-old boy with severe FD and a rapidly expanding femoral lesion. Immunohistochemical staining on a pretreatment bone biopsy showed marked RANKL expression. The patient was started on monthly denosumab, with an initial starting dose of 1- and 0.25-mg/kg dose escalations every 3 months.

Results: Over 7 months of treatment the patient showed marked reduction in pain, bone turnover markers, and tumor growth rate. Denosumab did not affect healing of a femoral fracture that occurred while on denosumab treatment. After treatment onset the patient developed hypophosphatemia and secondary hyperparathyroidism, and needed supplementation with phosphorus, calcium and calcitriol. Bone turnover markers showed rapid and sustained suppression. However, with discontinuation of denosumab there was a rapid and dramatic rebound of bone turnover markers, with the resorption marker CTX (reflecting osteoclast activity) exceeding pretreatment levels, and severe hypercalcemia.

Conclusion: Denosumab led to dramatic reduction of FD expansion and bone pain. However, the medication was associated with clinically significant disturbances in mineral metabolism both while on treatment and after discontinuation. Denosumab treatment of FD warrants further study to confirm efficacy and safety.

Fibrous dysplasia (FD) is a relatively common skeletal disorder in which normal bone is replaced by fibro-osseous tissue, leading to fracture, functional impairment, deformity and pain. FD may be isolated or occur in association with cutaneous hyperpigmentation and hyperfunctioning endocrinopathies. FD in combination with one or more extraskeletal manifestations is termed McCune-Albright syndrome (MAS). The treatment of endocrinopathies in MAS may be challenging. However, the often widely spread and expanding polyostotic skeletal lesions may cause even greater challenges, as in the patient reported here. Bisphosphonates have been used in the treatment of childhood-onset FD but especially in the polyostotic forms the efficacy is often questionable and is limited to relief of FD-related pain [10]. Against this background, denosumab, a fully-humanized monoclonal RANKL antibody, is a tempting therapeutic alternative. RANKL is expressed by osteogenic cells and plays a key role in osteoclastogenesis. By binding to its receptor on osteoclast progenitor membranes, RANKL promotes osteoclast differentiation and leads to increased bone resorption. RANKL inhibition via denosumab has recently been shown to be effective in the treatment of giant cell granulomas of bone, which like FD are derived from bone marrow stromal cells [11]. The treatment response in this patient was very promising as there was a marked reduction in the size of FD lesions, bone turnover markers and in bone pain. However, there were significant disturbances in mineral homeostasis after treatment cessation. Two months after the last infusion the patient developed severe symptomatic hypercalcemia (S-Ca 4.5 mmol/l); bone turnover markers were extremely elevated. With hydration, intravenous pamidronate and calcitonin the disturbances slowly normalized within 5 months. This apparently first case description of denosumab treatment in the pediatric age group suggests that this new medication may be efficacious in the treatment of severe polyostotic FD. At the same time it calls for caution when considering denosumab in children: the withdrawal effects may be unpredictable and exceptionally severe.

A novel tyrosine kinase inhibitor restores chondrocyte differentiation and promotes bone growth in a gain-of-function *Fgfr3* mouse model

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Background: Novel tyrosine kinase inhibitors are small molecular weight inhibitors of tyrosine phosphorylation that specifically inhibit the activity of tyrosine kinases, e.g. epidermal growth factor receptor (EGFR), fibroblast growth factor receptors (FGFRs), and the insulin receptor. Members of this new class of pharmaceuticals have shown to be useful in the treatment of a variety of malignancies. The specific inhibition of subclasses of receptors raises the hope that specific treatments for other conditions caused by increased tyrosine phosphorylation may be within reach. Along this line, the authors tested the hypothesis that a novel tyrosine kinase inhibitor targeting the increased intracellular signaling of FGFR3 may normalize the growth and growth plate histology of mice with an activating mutation in the FGFR3, the ‘achondroplasia mice’.

Methods: The authors used an ex-vivo culture model of femurs from *Fgfr3*(Y367C/+) dwarf and wild-type mice to study the effect of a novel tyrosine kinase inhibitor, A31, selected for its inhibitory effect on FGFR3 signaling.

Results: In their model system, A31 was able to normalize growth plate morphology, chondrocyte differentiation, proliferation, cell cycle regulator expression, as well as growth of cultured achondroplasia-mice femurs.

Conclusion: The findings suggest that specific tyrosine kinase inhibitors that target FGFR3 may provide an efficacious treatment of FGFR3-related chondrodysplasias.

In order to find a treatment for achondroplasia, several strategies aimed at reducing the excessive activity of FGFR3 have been proposed, including strategies to inhibit FGFR3 tyrosine kinase activity, to promote its degradation, or to antagonize its downstream signaling [12, 13]. In this article the authors show proof-of-concept for an appealing approach; inhibition of FGFR3 tyrosine kinase activity using a small molecular FGFR3 tyrosine kinase inhibitor. The authors find that growth and histology of ex-vivo cultured achondroplasia-mice femurs can be normalized by exposure to this specific inhibitor. This is an in-vitro finding and it is very possible that this finding may not be possible to replicate in vivo due to lack of efficient kinase inhibition or unacceptable side effects from other tyrosine kinases. Nevertheless, the finding that it is possible to reverse the effects of a constitutively active FGF receptor in cultured femurs makes it plausible that an inhibitor that is sufficiently specific to reverse the bone phenotype in vivo could be developed. Tyrosine kinase inhibition may thus be the most promising strategy currently being pursued in the quest for a specific treatment for achondroplasia, the most common form of skeletal dysplasia in humans.

Ablation of the proapoptotic protein Bax protects mice from glucocorticoid-induced bone growth impairment

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Background: Glucocorticoid (GC)-induced growth suppression is a common adverse effect during treatment of inflammatory disorders and malignancies. Several approaches, including growth hormone and IGF1 treatment, have been used to mitigate this unwanted side effect of GC treatment. In this experimental study, the authors use a different approach, targeting the proapoptotic gene Bax to test the hypothesis that Bax deficiency will prevent GC-induced apoptosis of growth plate chondrocytes and thereby prevent GC-induced growth impairment.

Methods: The authors used a combination of in-vitro and in-vivo studies including mice with targeted deletion of Bax to test their hypothesis.

Results: Dexamethasone activated Bax in chondrocytes by inducing a conformational change, resulting in its translocation to the mitochondria, and thus activating the intrinsic apoptotic pathway. Conversely, silencing of Bax blocked dexamethasone-induced apoptosis. Finally and most importantly, Bax-deficient female mice exhibited a near-normal growth rate, and were protected against glucocorticoid-induced bone growth impairment.

Conclusion: The findings suggest that the proapoptotic protein Bax is an important mediator of the growth-inhibiting effects of GC treatment in growth plate chondrocytes. Targeting of Bax may therefore be a possible strategy to circumvent the negative effect of GC treatment on longitudinal bone growth.

Glucocorticoids continue to be a widely used anti-inflammatory agent. In children, long-term use causes severe skeletal side effects, including growth retardation through multiple mechanisms including direct inhibition of growth plate chondrocyte proliferation, increased apoptosis, and likely also indirectly via effects on the GH/IGF-1 axis. Preventive measures targeting GC-induced apoptosis of growth plate chondrocytes appear particularly appealing since apoptosis may deplete the pool of resting and/or proliferative chondrocytes and, if so, would negatively affect the potential for future catch-up growth. The authors show that the growth-inhibiting effect of dexamethasone is prevented in Bax-deficient mice. The study does not address the effect of Bax deficiency on the desired, anti-inflammatory effects of GC treatment. If, in fact, Bax targeting does reverse some of the negative effects of GC treatment in humans without abolishing the anti-inflammatory effects, Bax targeting, presumably by small molecules or peptides, might turn out to be an attractive therapeutic approach to a common problem in children with chronic autoimmune conditions requiring long-term GC treatment. However, as we know, it is a long way from a knockout mouse to the growing child.

Role of fibroblast growth factor 21 (FGF21) in undernutrition-related attenuation of growth in mice

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Background: Insufficient caloric intake is known to inhibit skeletal growth, but the underlying hormonal/molecular mechanisms are not fully understood. Short-term caloric restriction is associated with reduced hepatic GH sensitivity as well as increased serum levels of FGF21. The authors explore the hypothesis that increased FGF21 activity during caloric restriction may result in reduced GH sensitivity and thereby contribute to the decrease in growth rate that occurs during food restriction.

Methods: The authors created FGF21 knockout mice (*fgf21* KO). These mice were subjected to 4 weeks of food restriction and the effect of FGF21 deficiency on growth inhibition and liver GH sensitivity was assessed.

Results: After the period of food restriction, *fgf21* KO mice exhibited greater body and tibial growth than wild-type (wt) littermates. Daily injections of recombinant human FGF21 in food-restricted *fgf21* KO mice prevented these differences. GH binding and GH receptor expression were reduced in the liver and in the growth plate of food-restricted wt mice (compared to wt mice fed ad libitum), whereas they were similar between food-restricted and ad libitum fed KO mice. In addition, a single injection of GH induced greater liver signal transducer and activator of transcription 5 phosphorylation and IGF-I mRNA in food-restricted KO mice than in wt mice. Lastly, in the tibial growth plate of food-restricted wt mice, FGF21 mRNA and protein expression was greater than that of wt mice fed ad libitum.

Conclusion: The findings suggest that inhibition of growth plate chondrogenesis and longitudinal bone growth during periods of low caloric intake is, in part, due to increased FGF21 activity and that this effect is due to antagonistic effect of FGF21 on GH action in the liver. In addition, the findings of FGF21 expression in growth plate cartilage and its upregulation during food restriction may suggest that FGF21 not only acts to regulate growth in an endocrine manner, but also may act locally to modulate growth.

FGF21 has extensively been reviewed in the *Yearbook* series and by others as a metabolic hormone with glucagon-like effects that is secreted from the liver in response to extended fasting [14]. FGF21 also acts in an autocrine fashion in several tissues, including adipose. The growth inhibition of food restriction is generally assumed to be mediated via the GH-IGF-I axis. In most mammals, fasting is associated with reduced IGF-I levels and reduced GH sensitivity in the liver. However, little is known of the molecular mechanism of this phenomenon. In this article, the authors show evidence that the upregulation of FGF21 expression during malnutrition may induce GH insensitivity in the liver. In addition, the finding of increased expression of FGF21 in growth plate cartilage suggests that FGF21 may also inhibit growth directly at the growth plate through the growth-inhibiting capacity of FGF receptors 1 and 3, expressed on proliferative and hypertrophic growth plate chondrocytes. These are interesting findings that provide a mechanistic explanation to the growth retardation that occurs during fasting or food restriction and may explain a common clinical problem in pediatric patients.

SOCS2 is the critical regulator of GH action in murine growth plate chondrogenesis

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Background: GH acts locally on the growth plate to promote bone growth. Suppressor of cytokine signaling-2 (SOCS2) is a negative regulator of GH signaling and bone growth via inhibition of the JAK/STAT pathway. Consequently, SOCS2 targeting in mice (SOCS2^{-/-}) results in increased longitudinal bone growth. The authors have previously demonstrated that this effect is due to loss of SOCS2 inhibition of GH signaling. In this follow-up study the authors clarify the mechanisms for the SOCS2-mediated inhibition of GH signaling in the growth plate.

Methods: The authors used wild-type and SOCS2^{-/-} mice and a combination of in-vitro and in-situ models to study the role of SOCS2-mediated inhibition.

Results: In SOCS2^{-/-} mice longitudinal bone growth rates, growth plate widths and chondrocyte proliferation are increased. The authors clarified the molecular mechanism for this finding as they showed that SOCS2, in contrast to SOCS1 and SOCS3 expression, was increased in cultured chondrocytes following GH challenge and that GH stimulated chondrocyte STATs-1, -3 and -5 phosphorylation in SOCS2^{-/-} but not in SOCS2 overexpressing cells. In addition, GH exposure of fetal metatarsal organ cultures stimulated growth of SOCS2^{-/-} but not wild-type bones. The stimulatory effect of GH on SOCS2^{-/-} metatarsal growth was blocked by a PI3K inhibitor, suggesting that this effect of GH is mediated through increased IGF1.

Conclusions: SOCS2 is crucial in modulating the local effects of GH on chondrocyte STAT activation, chondrocyte proliferation, and bone growth.

GH exerts most of its growth-promoting effect through IGF1, but it also stimulates growth by a direct action at the growth plate. In contrast to IGF1, the direct action of GH is frequently nonsignificant when assessed by in-vitro models of growth plate chondrogenesis, i.e. cultured chondrocytes or growth plate or whole-bone organ cultures. Interestingly, cultured metatarsal bones from SOCS2-deficient mice respond to GH with a solid increase in growth rate. However, as the authors show, this local effect of GH is mediated mostly through local production of IGF1, thus supporting the hypothesis that most of the growth-promoting effects of GH are mediated by IGF1.

Mechanism of the year It's all in the CYP genes

Mutations in CYP24A1 and idiopathic infantile hypercalcemia

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Background: In Britain in the 1950s there was increased incidence of idiopathic infantile hypercalcemia during a period of high vitamin D supplementation in fortified milk products. The authors investigated the molecular basis of idiopathic infantile hypercalcemia, which is characterized by severe hypercalcemia, failure to thrive, vomiting, dehydration, and nephrocalcinosis.

Methods: A candidate-gene approach was applied in a cohort of familial cases of idiopathic infantile hypercalcemia with suspected autosomal recessive inheritance. Identified mutations were evaluated with the use of a mammalian expression system.

Fig. 2. Vitamin D undergoes 25-hydroxylation in the liver and 1 α -hydroxylation in the kidney, resulting in the formation of active 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃), which binds to the vitamin D receptor (VDR). The 1,25-(OH)₂D₃ is catabolized by CYP24A1 to water-soluble calcitroic acid. The enzymatic activities of CYP27B1 and CYP24A1 are tightly controlled by the levels of 1,25-(OH)₂D₃, serum calcium, and parathyroid hormone. In addition, 1,25-(OH)₂D₃ exerts negative feedback on CYP27B1 through fibroblast growth factor 23 (FGF23) and Klotho.

Results: Sequence analysis of *CYP24A1*, which encodes 25-hydroxyvitamin D 24-hydroxylase, the key enzyme of 1,25-dihydroxyvitamin D degradation, revealed recessive mutations in 6 affected children. Subsequently, *CYP24A1* mutations were identified in a cohort of infants who developed severe hypercalcemia after bolus prophylaxis with vitamin D. Functional characterization revealed a complete loss of function in all *CYP24A1* mutations.

Conclusion: The *CYP24A1* mutations explain the increased sensitivity to vitamin D in patients with idiopathic infantile hypercalcemia and are a genetic risk factor for hypercalcemia during vitamin D prophylaxis in otherwise healthy infants.

The authors studied a cohort of 6 patients from four families with idiopathic infantile hypercalcemia – an enigmatic condition so far. The inheritance pattern suggested an autosomal recessive condition. A second cohort consisted of 4 patients with suspected vitamin D intoxication in whom severe hypercalcemia developed after bolus prophylaxis with vitamin D. The authors looked for mutations in four candidate genes that encode key enzymes involved in vitamin D metabolism: *CYP24A1*, *CYP27B1*, *FGF23* and *KL* (encoding Klotho) (fig. 2). In both cohorts, loss-of-function mutations in the *CYP24A1* gene were identified. These abolished the enzyme's capacity to degrade 1,25-dihydroxyvitamin D into 24,25-dihydroxyvitamin D, thereby rendering the subjects susceptible to vitamin D toxicity. The study by Dauber et al. [15] took another approach with a patient with severe infantile hypercalcemia. The patient presented at the age of 10 months with hypercalcemia, suppressed PTH, hypercalciuria, nephrocalcinosis and normal 25-OH and 1,25-OH₂ vitamin D levels. Whole-exome sequencing of the patient's DNA identified a homozygous 3-bp in-frame deletion in the *CYP24A1* gene. In accordance with this, the serum levels of 24,25-dihydroxyvitamin D were undetectable. Streeten et al. [16] described hypercalcemia in an adult patient with a homozygous loss-of-function mutation in the same gene. Interestingly, in the report by Dauber et al. [15] the patient's mother, a heterozygous mutation carrier, had nephrolithiasis. This may suggest hypercalciuria and presence of a carrier phenotype. These reports together provide solid evidence for the causative role of *CYP24A1* mutations in idiopathic hypercalcemia. However, several questions remain unanswered: What is the natural course of hypercalcemia as some probands became asymptomatic with time while others only developed symptoms at adult age? What is the mechanism behind hypercalcemia? Probably what counts is the intracellular 1,25-dihydroxyvitamin D concentration, as the measured circulating 1,25-dihydroxyvitamin D levels tended to be normal. And finally, do the heterozygous mutation carriers have normal calcium homeostasis? In any case, this study opens a new view to infantile hypercalcemia.

Enzyme-replacement therapy in life-threatening hypophosphatasia

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Background: Hypophosphatasia results from mutations in the *TNSALP* gene encoding the tissue-nonspecific isozyme of alkaline phosphatase. Consequent accumulation of inorganic pyrophosphate leads to rickets or osteomalacia. Severely affected infants often die from respiratory insufficiency or have persistent bone disease. ENB-0040, a bone-targeted recombinant human TNSALP, prevents the manifestations of hypophosphatasia in *Tnsalp* knockout mice.

Methods: The authors carried out a multinational, open-label study of treatment with ENB-0040 in infants and young children with life-threatening or debilitating perinatal or infantile hypophosphatasia. The primary objective was the healing of rickets, as assessed radiographically. Motor and cognitive development, respiratory function, and safety were evaluated, as well as the pharmacokinetics and pharmacodynamics of ENB-0040.

Results: Of the 11 patients recruited, 10 completed 6 months of therapy and 9 completed 1 year. At 6 months the authors observed healing of rickets and improvement in developmental milestones and pulmonary function. Plasma levels of inorganic pyrophosphate and pyridoxal 5'-phosphate diminished. Increases in serum parathyroid hormone accompanied skeletal healing, often necessitating dietary calcium supplementation. There was no evidence of hypocalcemia, ectopic calcification, or serious adverse events.

Conclusion: ENB-0040, an enzyme-replacement therapy, significantly improved radiographic abnormalities and pulmonary and physical function in infants and young children with life-threatening hypophosphatasia.

Hypophosphatasia is characterized by defective skeletal mineralization. It ranges in severity from neonatally lethal forms with almost entirely absent mineralization to late-onset mild forms with minor skeletal or dental problems. Attempts to normalize mineralization with intravenous infusions of plasma enriched in soluble alkaline phosphatase have been unsuccessful. The present study reports preliminary results of an open-label study with an investigational recombinant enzyme-replacement therapy in 11 patients with life-threatening perinatal or infantile hypophosphatasia. All patients were younger than 3 years of age at study onset. The study preparation was given as one initial intravenous infusion followed by subcutaneous injections three times per week. Ten of the 11 patients completed the 6-month study period and continued treatment in the study extension. The enzyme-replacement therapy resulted in a significant improvement of radiological signs, showing improved mineralization, bone growth and normalization of bone shape. This was translated to improved respiratory function, mobility and well-being. Side effects were scarce and the treatment was overall well tolerated. The paper carefully documents the major biochemical, radiographic and clinical outcome measures in the study cohort. The findings are extremely important and interesting and provide hope for children with this life-threatening disease. This study, however, has to be seen as a preliminary short-term pilot study and more studies and long-term follow-up data are needed to confirm the positive results. Further studies will hopefully provide more solid ground for optimism. The unavoidable question in the near future will be where to draw the line when deciding about the therapy target group – a much larger patient population suffers from milder forms of hypophosphatasia and although not life-threatening, the disease is severely incapacitating. Will this new treatment be available to them too?

Long-term higher urinary calcium excretion within the normal physiologic range predicts impaired bone status of the proximal radius in healthy children with higher potential renal acid load

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Background: Reduced bone mineral density (BMD) has been observed in children with idiopathic hypercalciuria. The authors evaluated whether higher urinary calcium excretion within the normal physiologic range influenced bone characteristics in healthy children.

Methods: Urinary calcium excretion was quantified in 603 24-hour urine samples from 154 healthy children and adolescents, and parallel 3-day weighed dietary records were analyzed repeatedly during the 4 years preceding evaluation of proximal forearm bone status by peripheral quantitative computed tomography (pQCT). Urinary potential renal acid load (uPRAL) was determined according to urine ionogram by subtracting measured quantitatively important mineral cations from nonbicarbonate anions.

Results: Urinary calcium excretion was significantly associated with volumetric BMD ($p = 0.04$), almost significantly with cortical bone mineral content (BMC) ($p = 0.05$), but not with bone cross-sectional area (CSA), or strength-strain index. When the analyses were stratified by uPRAL, calcium excretion was negatively associated with volumetric BMD ($p = 0.007$), cortical BMC ($p = 0.001$), and cortical CSA ($p = 0.004$) in those children with higher uPRALs, but not in those with low uPRALs ($p > 0.3$).

Conclusion: Long-term higher calciuria within the physiological range predicts reduced diaphyseal bone mass and bone density particularly in children with long-term unfavorable higher dietary acid load, i.e. with lower fruit and vegetable intake.

While several studies have explored the influence of calcium intake on skeletal characteristics, this study takes a look at the output, namely urinary calcium excretion. This is impacted by the intake, but not solely by calcium intake but also by salt and protein intake and dietary acid load. Genetic factors also play a role. This meticulously performed longitudinal study in 6- to 18-year-old children evaluated the impact of urinary calcium excretion, within the normal physiologic range, on bone health. Those with supranormal urinary calcium excretion (exceeding 4 mg or 0.1 mmol/kg body weight) were excluded. Dietary intakes during the 4 preceding years were collected, parallel to 24-hour urine samples, to determine the degree of calcium excretion. The skeletal characteristics were determined by pQCT of the nondominant forearm. The authors observed significant differences in urinary potential renal acid load (uPRAL) but this alone did not associate with any of the pQCT-derived bone parameters. In contrast, urinary calcium excretion was a significant determinant of volumetric bone density especially when diet acid load was high: calcium excretion demonstrated significant negative associations with cortical bone mineral content, cortical cross-sectional area and cortical volumetric BMD in subjects belonging to the highest tertile of uPRAL. Urine calcium excretion was most significantly determined by sodium and nitrogen excretion, dietary acid load and by growth velocity, and less so by dietary calcium intake. Furthermore, there was no positive association between calcium intake and diaphyseal bone status, challenging the idea that increased dietary calcium consumption has consistent beneficial effects on bone health. The present study shows that several other factors need to be considered in order to optimize bone health during childhood and adolescence. The diet-independent urinary calcium excretion is important for bone health even when within the physiologic range. A long-term reduction in dietary acid load may have a positive overall effect on bone health. This can be obtained by high fruit and vegetable intake, which provides alkaline salts to compensate for the high acid load from protein-rich Western diet. It seems that this study, as so many others in various areas of medicine, comes to the same conclusion – more fruits and vegetables for all of us!

25-Hydroxyvitamin D assay variations and impact on clinical decision-making

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Background: Several new automated 25-hydroxyvitamin D (25-OHD) assays are available. These methods often show variability in results.

Methods: The authors measured 25-OHD levels in 494 patients using two different methods, Immunodiagnostic Systems RIA (IDS-RIA) and DiaSorin Liaison assays. Sources of variability between the assays were investigated in 83 samples that were retested in the US reference laboratory, and by reviewing the performance reports issued by the International Vitamin D External Quality Assessment Scheme, DEQAS.

Results: The Liaison method gave significantly lower values than IDS-RIA. The mean bias was -5 ng/ml (range -38.1 to $+18.7$ ng/ml, $p < 0.001$); the absolute difference was independent of 25-OHD value. Interassay variability was also detected in values obtained in the reference laboratory and in DEQAS reports. The values impacted clinical decision-making regarding vitamin D treatment. Using 20 ng/ml as the target 25-OHD level, 52% required treatment when tested by Liaison and 36% when tested by IDS-RIA ($p < 0.001$). Using 30 ng/ml as the desirable level, the proportions were 79 and 64%, respectively ($p < 0.001$). The two assays agreed in only 41–68% of the subjects.

Conclusion: The 25-OHD assays have a significant impact on the results, patient classification, and treatment recommendations. Such variability should not be ignored when determining and applying vitamin D guidelines. Universal assay standardization is urgently needed.

Approximately 1 billion people worldwide are suspected to have vitamin D deficiency or insufficiency. Vitamin D deficiency has been associated with not only musculoskeletal health but also with cancer, cardiovascular disease, diabetes, and autoimmune disorders. Serum 25-OH-vitamin D (25-OHD) is widely used as a marker for vitamin D status and to guide replacement needs. Controversy exists regarding the optimal target level of serum 25-OHD. Recent recommendations include a target >20 ng/ml (50 nmol/l) by the Institute of Medicine [17] or 30 ng/ml (75 nmol/l) by the International Osteoporosis Foundation [18] and The Endocrine Society 2011 Clinical Practice Guidelines [19]. In children, 50 nmol/l is generally regarded as the lower limit for vitamin D sufficiency [20]. However, these guidelines are of little value if the methodology whereby we determine 25-OHD status is not valid. In this paper the researches quantified the sources of variability in 25-OHD measurements that occurred during a shift in assays from a traditional RIA to a more rapid automated assay. In IDS-RIA the 25-OHD concentration is inversely proportional to the bound radioactivity whereas DiaSorin Liaison uses chemiluminescent immunoassay. In the cohort of 494 patients the two methods gave consistently different results with an interassay CV of $20.6 \pm 14.2\%$; the IDS-RIA values were on average 5 ng/ml (12.5 nmol/l) higher (range -38.1 to $+18.7$ ng/ml, or -95.2 to $+46.7$ nmol/l). These differences had a significant influence on clinical decision-making (fig. 3). Clinicians should be aware of this variability and stay alert about not only the results but the methodology behind the measured 25-OHD values. With a huge fraction of children anywhere having vitamin D insufficiency, do we really need to measure 25-OHD on every child? And even more so when the assay is only partly reliable. D is a vitamin; it needs to be added in the diet or taken as a supplement.

Fig. 3. Proportion of subjects by serum 25-OHD level in the study cohort using the DiaSorin Liaison and the IDS-RIA assays. S-25-OHD 20 ng/ml corresponds to 50 nmol/l and 30 ng/ml to 75 nmol/l.

Concepts revised

What is vitamin D really needed for?

Calcium absorption, kinetics, bone density, and bone structure in patients with hereditary vitamin D-resistant rickets

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Background: Hereditary 1,25-dihydroxyvitamin D-resistant rickets (HVDRR), caused by mutations in the vitamin D receptor gene, results in severe hypocalcemia and rickets and in childhood requires treatment with high-dose calcium supplements. Surprisingly, calcium metabolism recovers postpubertally. This study evaluated the role of vitamin D receptor in intestinal fractional calcium absorption (FCA), bone calcium accretion, bone mineral density (BMD), and bone structure from infancy to adulthood.

Methods: The authors investigated 17 HVDRR patients aged 1.5–37 year. FCA and bone calcium accretion were determined by stable calcium isotopes; BMD was assessed by dual-energy x-ray absorptiometry and bone structure by high-resolution magnetic resonance imaging.

Results: FCA in patients aged 1.5–17 years was significantly lower than in age-matched controls ($p < 0.00004$), whereas in patients aged 18–26 years, it was higher than in controls ($p < 0.001$). In patients older than 29 years FCA was comparable to controls. Patients aged 18–26 years had higher bone calcium accretion than controls ($p < 0.02$) while bone calcium accretion in patients under 18 and over 29 years of age did not differ from controls. Femoral-neck BMD Z-score was -2.38 ± 0.3 in patients under 18 years and 0.28 ± 0.87 in postpubertal patients ($p < 0.0001$). High-resolution magnetic resonance imaging showed similar bone structure and bone parameters in HVDRR patients and controls.

Conclusion: These findings in HVDRR patients suggest that calcium absorption is highly vitamin D-dependent from infancy until the end of puberty, after which other mechanisms, independent of vitamin D, are substantially involved in calcium absorption.

Monogenic human disorders provide unique models to study physiology. Here the authors have carefully evaluated a relatively large cohort of patients with a rare recessive disorder in which loss-of-function mutations in the vitamin D receptor (VDR) gene render the receptor unresponsive to $1,25-(\text{OH})_2\text{D}$. Vitamin D resistance results in severe rickets, hypocalcemia, hypophosphatemia, elevated PTH and $1,25-(\text{OH})_2\text{D}$, and in alopecia. During infancy and childhood of these patients, extremely high doses of

calcium are needed to control hypocalcemia and rickets. However, calcium requirements spontaneously normalize after puberty. This can be explained by lower calcium demand after cessation of growth and by activation of VDR-independent mechanisms of calcium absorption postpubertally. Intestinal calcium absorption and bone calcium accretion, studied with stable calcium isotopes, showed unique profiles. Postpubertally, intestinal calcium absorption increased to levels exceeding those in the controls. Studies in VDR knockout mice have shown that during pregnancy and lactation VDR-independent intestinal calcium absorption and skeletal mineralization are upregulated; this may be mediated by placental lactogen and prolactin. The present study shows that such mechanisms are activated also at the end of puberty, suggesting that sex hormones may stimulate intestinal calcium transport. The findings raise several questions that can be only partly answered based on the available data. Firstly, what are the VDR-independent intestinal mechanisms for calcium absorption and how are they regulated by various hormones and at different stages of growth and puberty. Interestingly, the gastric mucosa in male and female adult rats has a substantial aromatase activity [21] and if present also in humans, suggests a role for estrogen in this process. Secondly, it is intriguing that skeletal characteristics in these individuals with complete 1,25-(OH)₂D resistance showed no difference from healthy controls in young adulthood. The question arises, what indeed is the role of vitamin D in the skeletal development and homeostasis? And lastly, what is the overall health outcome of these individuals who are deprived from all the nonskeletal benefits of vitamin D because of a receptor problem? There is much more to be learnt.

Reviews

Bones – connecting people

The contribution of bone to whole-organism physiology

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Background: The mouse genetic revolution has shown repeatedly that most organs have more functions than expected. To uncover these, there is a need for a whole-organism study of physiology.

Methods: This paper reviews the current knowledge on the interactions between bone and other organ systems.

Results: Moving from molecular and cellular approach to whole-organism studies in the evaluation of skeletal physiology has broadened our understanding of the various skeletal functions and revealed important connections between bone and other organs such as the brain, pancreas and gut.

Conclusions: A whole-organism approach is needed to understand the physiological role of the skeleton. This approach is likely to shed new light on the pathogenesis of degenerative diseases affecting multiple organs.

Recent years have significantly broadened our view about skeletal functions. Some of these new discoveries were highlighted in last year's *Yearbook* [1]. The key molecule mediating signals from the skeleton to other organs is osteocalcin, an osteoblast-specific molecule, which has a function in the regulation of whole-body glucose homeostasis and energy expenditure [22, 23]. Furthermore, osteocalcin regulates male fertility [24]. In this review the authors summarize the details of these and other connections as they are presently understood. However, the authors conclude that if so much has been learned in such a short time about the physiology of one organ, it is likely that several other aspects will be discovered with similar whole-organism approach in the near future. This approach is based on the ideas that firstly, no body function is determined by one organ alone, and second, different organs exert opposite influences on the same function to regulate it tightly and maintain homeostasis. Furthermore, the cardinal rule of endocrinology applies to most systems: a regulated organ talks back to another regulating one to limit its influence. Interestingly, the bone seems to play a key role in the communication and networking with other organs.

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