Growth Plate, Bone and Calcium

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Studies of bone biology have been complicated by the highly organized structures and complex cellular biology. Thanks to new models and technologies, our view and understanding of the biological importance of the skeleton has expanded dramatically in the last years. We first take the opportunity to highlight a few papers discussed in this chapter. The recent discovery of the skeleton as an endocrine regulator of energy metabolism was chosen as the **mechanism of the year**. Two papers coupling bone formation and angiogenesis and another elegant study identifying a nucleotide-sugar transporter to be crucial for normal skeletal development represent **new paradigms**. The possibility to use stem cells as a future tool to repair bone defects gives **new hope** for the therapeutic intervention of bone disorders. An important **observation for clinical practice** is that serum measurements of fibroblast growth factor 23 (FGF23) can be used as a clinical diagnostic tool in the workup of patients with hypophosphatemia. A recent publication of age-, race-, and sex-specific pediatric reference data for bone mineral content/density is of great **importance for clinical practice and research**. We also included a clinical study indicating that calcimimetics may offer a **new treatment** option in patients with hypophosphatemic rickets reducing the risks of complications in these patients. Two papers describing patients with de novo mutations resulting in Klotho excess and deficiency, respectively, have revealed a **new mechanism** of Klotho action in human phosphate homeostasis. In the section **new genes**, a paper identifying multiple genetic loci for bone mineral density and fractures is discussed. Two **reviews** that should not be missed are also highlighted; first a paper entitled 'Vitamin D deficiency: a worldwide problem with health consequences' followed by a paper presenting a unifying new somatomedin hypothesis entitled 'The somatomedin hypothesis 2007: 50 years later'.

Mechanism of the year Bones play a role in diabetes

Endocrine regulation of energy metabolism by the skeleton

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Cell 2007;130:456–469

Background: Obesity has been shown to protect mammals from osteoporosis. The authors have previously demonstrated that leptin, an adipocyte-derived hormone is a major regulator of bone remodeling by acting on osteoblasts through two different neural pathways. They furthermore hypothesized that bone may exert a feedback control of energy homeostasis.

Methods: To test this hypothesis the authors searched for genes expressed in osteoblasts, encoding signaling molecules and affecting energy metabolism. They generated and studied the phenotypes of both osteoblast-targeted knockout mice lacking the *Esp* gene (encoding tyrosine phosphatase OST-PTP) and osteocalcin. Multiple different metabolic studies were performed both in vivo and in vitro.

Results: The authors demonstrated that mice lacking the protein tyrosine phosphatase OST-PTP were hypoglycemic and protected from obesity and glucose intolerance. This was due to an increase in β -cell proliferation, insulin secretion, and insulin sensitivity. In contrast, mice lacking the osteoblast-secreted molecule osteocalcin displayed decreased β -cell proliferation, glucose intolerance, and insulin resistance.

Removing one *Osteocalcin* allele from OST-PTP-deficient mice corrected their metabolic phenotype. Ex vivo, osteocalcin was able to stimulate *CyclinD1* and *Insulin* expression in β -cells and adiponectin in adipocytes. Furthermore, osteocalcin was able to improve glucose tolerance in vivo.

Conclusion(s): This study revealed that the skeleton exerts an endocrine regulation of sugar homeostasis. Our view and understanding of the biological importance of the skeleton has now taken a big step forward.

An interaction of energy metabolism and the bone was suggested some years ago by demonstrating that leptin is a major regulator of bone remodeling [1]. We have also learned that osteoblasts and preadipocytes originate from the same stem cell. This concept is now being expanded. Immediately after its publication, this paper by Lee et al. was discussed in science news around the world. The concept of the skeleton functioning as part of the endocrine system and playing a role in regulating energy metabolism was completely new. Luckily for investigators in the field, there are only a few cell-specific genes that are expressed in bone, and osteocalcin is the most prominent of them. It has now become a hormone, circulating from bone to pancreas. By using several different mouse models and experiments, the authors showed that osteocalcin, a protein secreted by osteoblasts and osteocytes, regulates insulin production and insulin sensitivity in the body, shedding light on a long-standing question about the function of osteocalcin. Karsenty and colleagues first created osteocalcin knockout mice in 1996. They noted that the mice were fatter than normal, but at the time they did not measure changes in blood sugar or other markers of energy metabolism. In the current study, the authors carefully examined osteocalcin and *Esp* knockout mice. *Esp*^{-/-} mice were hypoglycemic, had decreased adiposity and improved insulin sensitivity, and were resistant to obesity and glucose intolerance. Surprisingly, the metabolic phenotype of *Osteocalcin*^{-/-} mice was a mirror image of the *Esp*^{-/-} phenotype. Despite being fed a normal diet, *Osteocalcin*^{-/-} mice had increased glucose levels and decreased insulin sensitivity, and developed type 2 diabetes. Mice overexpressing osteocalcin showed the opposite characteristics. Osteocalcin stimulated pancreatic β cells to produce insulin and promoted the growth of new β cells in vitro, and also stimulated fat cells to produce adiponectin, a metabolic hormone that regulates insulin sensitivity. Because bone is undergoing constant remodeling, it makes sense that the skeletal system must communicate with energy reserves. 'If fat speaks to bone, bone must speak to fat,' Gerald Karsenty said in an interview after the paper came out. 'The surprise was the finding that osteocalcin is the messenger.' The molecular mechanisms underlying this communication still remain unknown but the story is continuing: a more recent paper from the same group confirms the positive effects of osteocalcin on sugar homeostasis [2]. Why would a bone-specific hormone regulate energy metabolism, and what is the need for a bone hormone favoring β -cell proliferation and insulin secretion? It is only a while ago that the osteoblasts and osteocytes were discovered to produce another hormone – FGF23 [1, 3, 4]. Expect more surprises from this cell type.

New paradigms

Hints from HIF: coupling bone formation and angiogenesis

The hypoxia-inducible factor- α pathway couples angiogenesis to osteogenesis during skeletal development

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J Clin Invest 2007;117:1616–1626

Background: Bone development and turnover occurs in close association with angiogenesis. The bone-forming osteoblasts are ideally situated in bone to sense oxygen tension and respond to hypoxia by activating the hypoxia-inducible factor- α (HIF- α) pathway. The aim was to study the mechanisms coupling bone and vascular physiology.

Methods: The authors generated conditional von Hippel-Lindau (*Vhl*), *Hif1a* and double knockout mice. Skeletal phenotypes were analyzed by phenotyping, histology and in situ hybridization. Angiogenesis and bone formation were studied in vitro by metatarsal organ cultures and primary osteoblast cultures.

Results: Mice overexpressing HIF- α in osteoblasts through selective deletion of the *Vhl* gene expressed high levels of *Vegf* and developed extremely dense, heavily vascularized long bones. By contrast, mice lacking *Hif1a* in osteoblasts had the reverse skeletal phenotype. Their long bones were significantly thinner and less vascularized than those of controls. Loss of *Vhl* in osteoblasts increased endothelial sprouting from the embryonic metatarsals in vitro but had little effect on osteoblast function in the absence of blood vessels. Double knockout mice lacking both *Vhl* and *Hif1a* had an intermediate bone phenotype, suggesting overlapping functions of HIFs in bone.

Conclusion(s): Activation of the HIF- α pathway in the developing bone increases bone modeling events. This occurs via cell non-autonomous mechanisms to coordinate the timing, direction, and degree of new blood vessel formation in bone.

Activation of the hypoxia-inducible factor-1 α pathway accelerates bone regeneration

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Proc Natl Acad Sci USA 2008;105:686–691

Background: The authors previously demonstrated that the hypoxia-inducible factor-1 α (HIF-1 α) pathway is the central regulator of adaptive responses to low oxygen availability and is required for normal skeletal development. The aim of this study was to examine the possible role of the HIF-1 α pathway during bone repair, and whether this pathway could be genetically and pharmacologically manipulated to improve skeletal healing.

Methods: The authors used the same knockout animals as described in their previous paper. Distraction osteogenesis was performed on the animals and the bones were studied by radiology, biomechanical testing, histology, immunohistochemistry and in situ hybridization. The effects of pharmacological activation of the HIF-1 α pathway were studied in vitro and in vivo.

Results: Mice lacking the von Hippel-Lindau protein (pVHL) in osteoblasts with constitutive HIF-1 α activation had markedly increased vascularity. More bone was produced in response to distraction osteogenesis, whereas mice lacking HIF-1 α in osteoblasts had impaired angiogenesis and bone healing. The increased vascularity and bone regeneration in the *pVHL* mutants were vascular endothelial growth factor (VEGF)-dependent and eliminated by concomitant administration of VEGF receptor antibodies. Small-molecule inhibitors of HIF prolyl hydroxylation stabilized HIF/VEGF production and increased angiogenesis in vitro. One of these molecules (DFO) administered in vivo into the distraction gap increased angiogenesis and markedly improved bone regeneration.

Conclusion(s): The HIF-1 α pathway is a critical mediator of neoangiogenesis, which is required for skeletal regeneration. The application of HIF activators could be a useful therapy to improve bone healing.

These two elegant papers, which were published within 6 months by the same group, give us a better understanding of the mechanisms coupling bone formation and angiogenesis. The vasculature provides many important elements for successful bone formation, including a niche and source of both hematopoietic and mesenchymal stem cells, the organizational structure for haversian channels and the supply for calcium, phosphate and nutrients. However, very little is known about the integration of and signaling in vasculature during skeletogenesis. VEGF is a prototype of an osteogenic-angiogenic coupling factor that plays multiple roles in skeletal development, growth and repair. In the current papers, Clemens et al. establish critical roles for HIF-1 α and HIF-2 α (denoted as HIF- α below) in bone formation. They first demonstrated that osteoblasts express all the necessary components of the oxygen-sensing pathway, including HIF- α . They furthermore showed that the hypoxia-dependent HIF- α accumulation in the osteoblast nucleus was followed by up-regulation of VEGF expression. By Cre-lox technology, conditional osteoblast-targeted knockouts for both *Vhl* (leading to accumulation of HIF- α) and *Hif1a* (leading to lack of HIF-1 α) were created and the reverse bone phenotypes were found. The overlapping redundancy by remaining HIF-2 α activity in the latter phenotype was carefully confirmed

Fig. 1. Mechanisms for coupling of osteogenesis and angiogenesis. Osteoblastic HIF-1 α subunits are transcriptional regulators of VEGF expression. Under normoxic conditions, HIF-1 α is hydroxylated by prolyl hydroxylase (PHD) and hydroxylated subunits are bound by pVHL, which regulates their ubiquitination and destruction. Under hypoxic conditions, PHD is inhibited and HIF-1 α is translocated into the nucleus, where it up-regulates *Vegf* gene expression together with coactivators. Osteoblast-derived VEGF protein has stimulatory effects both on the differentiation of endothelial progenitors and directly on mature endothelial cells. Endothelial cells in turn can stimulate the differentiation of pericytes (also known as microvascular smooth muscle cells) into osteoprogenitors by secreting bone morphogenetic proteins (BMPs). However, the correct microenvironment including osteogenic signals from the bone marrow and extracellular matrix are also needed.

with a double knockout. In the other paper, the same group studied the effects of distraction osteogenesis in knockout mice, and demonstrated that *Vhl* knockouts had improved neoangiogenesis and bone regeneration, while disruption of *Hif1a* impaired the process of bone healing. Small molecules, which inhibit HIF prolyl hydroxylation thus activating the HIF-1 α pathway were tested both in vitro and in vivo. Angiogenesis and bone formation were increased by exposure to these compounds, suggesting that they could be promising agents to improve blood supply to skeletal tissues during regeneration. The present working model for osteogenic-angiogenic coupling via HIF- α and VEGF is summarized in figure 1. Regardless of the complex cellular mechanisms, these studies clearly show that HIF-dependent angiogenesis is a primary stimulus for osteoblast-mediated osteogenesis early during endochondral bone formation. The apparent requirement for angiogenesis in bone formation suggests the possibility that agents that stimulate angiogenesis might promote bone regeneration.

New paradigms

Nucleotide-sugar transporter critical for skeletal development

Nucleotide-sugar transporter SLC35D1 is critical to chondroitin sulfate synthesis in cartilage and skeletal development in mouse and human

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Nat Med 2007;13:1363–1367

Background: Proteoglycans have critical roles in chondrogenesis and skeletal development. The glycosaminoglycan chains found in cartilage proteoglycans are primarily composed of chondroitin sulfate.

The integrity of chondroitin sulfate chains is important to cartilage proteoglycan function; however, chondroitin sulfate metabolism in mammals remains poorly understood. The solute carrier-35 D1 (SLC35D1) gene (SLC35D1) encodes an endoplasmic reticulum nucleotide-sugar transporter (NST) that might transport substrates needed for chondroitin sulfate biosynthesis.

Methods: The authors created *Slc35d1*-deficient mice that develop a lethal form of skeletal dysplasia with severe shortening of limbs and facial structures. In addition, mutational analysis of the *Slc35d1* gene was performed in subjects with Schneckenbecken dysplasia, a severe skeletal dysplasia.

Results: Epiphyseal cartilage in homozygous mutant mice showed a decreased proliferating zone with round chondrocytes, scarce matrices and reduced proteoglycan aggregates. These mice had short, sparse chondroitin sulfate chains caused by a defect in chondroitin sulfate biosynthesis. In addition, the authors identified that loss-of-function mutations in human SLC35D1 cause Schneckenbecken dysplasia.

Conclusion(s): The findings highlight the crucial role of NSTs in proteoglycan function and cartilage metabolism thus revealing a new paradigm for skeletal disease and glycobiology.

Schneckenbecken dysplasia (OMIM 269250) is a perinatally lethal skeletal dysplasia that is inherited as an autosomal recessive trait. Its rarity does not preclude its importance for understanding basic mechanisms. The German term 'Schneckenbecken' refers to the distinctive, snail-like appearance of the ilia which results from a medial bone projection from the inner iliac margin. Other hallmarks of Schneckenbecken dysplasia include thoracic hypoplasia, severe flattening of the vertebral bodies and short, thick long bones. The similarities of the skeletal and histological findings in *Slc35d1*^{-/-} mice to the Schneckenbecken dysplasia phenotype prompted the authors to search for SLC35D1 mutations in individuals with Schneckenbecken dysplasia. In 2 subjects with typical Schneckenbecken dysplasia, mutations were identified in both alleles. The identification of the SLC35D1 mutation in Schneckenbecken dysplasia contributes to the molecular classification and diagnosis of perinatally lethal skeletal dysplasias. Human skeletal dysplasias are grouped by their phenotypic similarities, which reflect causal similarity in many cases. Schneckenbecken dysplasia belongs to the 'severe spondylodysplastic dysplasias' group, for which no causative mutations were known before this study. SLC35D1 and other genes acting in the same genetic pathway may well be responsible for these lethal skeletal dysplasias. This study may help build new bridges between skeletal biology and glycobiology.

New hope

Stem cells as a future tool to repair bone defects

Intrauterine transplantation of human fetal mesenchymal stem cells from first-trimester blood repairs bone and reduces fractures in osteogenesis imperfecta mice

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Blood 2008;111:1717–1725

Background: Osteogenesis imperfecta (OI) is an inherited skeletal dysplasia, which results in multiple fractures. The current empirical treatment for OI is the use of bisphosphonates, which does not address the underlying collagen defect.

Methods: The authors transplanted human first-trimester fetal blood mesenchymal stem cells (MSCs) into OI model (*oim/oim*) mice in utero. Fracture rate, bone histomorphometry and bone mechanical properties were studied.

Results: MSC transplantation resulted in a significant reduction of long bone fractures ($p < 0.01$) with fewer fractures per mouse. Nearly all mice that did not receive transplants had fractures (97.9%), in contrast to 4- to 12-week-old mice that received transplants (58.6%) ($p < 0.01$). Transplantation was significantly associated with increased bone strength, thickness and length. Growth plate height was also normalized in transplanted *oim/oim* mice ($p < 0.001$). Interestingly, more donor cells were found in bone tissues compared with other organs ($p < 0.001$) and MSCs clustered at sites of fracture healing

with active bone formation and remodeling. Donor cells found in the bone expressed genes for osteoblastic lineage, and produced the bone extracellular protein osteopontin. In addition, MSC transplantation decreased bone hydroxyproline content.

Conclusion(s): Intrauterine transplantation of fetal MSCs markedly reduced fracture rates and skeletal abnormalities in a mouse model of the intermediate severity type III OI, suggesting a scientific basis for MSC treatment of affected human fetuses.

OI is an inherited disease varying in severity from perinatally lethal type II to the non-deforming type I. Current treatment is based on the use of bisphosphonates to prevent fractures and on surgery (e.g. rodding of long bones and bracing of lower limbs). However, these treatments do not address the underlying collagen defects. In this paper, the authors demonstrated a significant reduction of fractures in a mouse model for OI (*oim/oim*) by in utero MSC transplantation. Amazingly, MSCs possess homing and engraftment capabilities for malfunctioning tissues. MSCs are non-hematopoietic clonogenic cells, which are capable of differentiating into e.g. cells of bone, cartilage, adipose and muscle tissues. Their multipotent nature makes them ideal candidates for tissue engineering. In this study, MSCs were obtained from human fetal blood, characterized and implanted into *oim/oim* fetuses on E13.5–E15. At each postnatal time point, the fracture rate of long bones was lower in transplanted compared to non-transplanted mice. Bone thickness, length and mechanical properties were also significantly increased, which are clinically relevant outcomes. The authors detected MSCs in various organs for up to 12 weeks after birth and interestingly, donor cell retention was greater in the skeleton compared to other organs, suggesting the presence of homing signals in bone. Furthermore, transplanted MSCs were clustered in areas of active bone turnover. The question remains how human cells are capable of having such therapeutic actions in mice, and whether a similar treatment could be used to benefit OI patients. Previously, Horwitz et al. transplanted bone marrow MSCs into three 3- to 17-month-old children with type III OI and demonstrated a 45–77% increase in bone mineral content [5]. Le Blanc and colleagues rescued an affected human fetus by in utero transplantation of fetal liver MSCs, although the latter outcome in fracture incidence was confounded by concomitant bisphosphonate therapy [6]. Clearly, MSCs possess a vast potential in both pre- and postnatal tissue regeneration. This piece of work takes us a step forward in the development of prenatal therapies for early-onset mesenchymal diseases. How about GH-insensitivity syndromes?

Important for clinical practice Serum FGF23 as a clinical tool?

Clinical usefulness of measurement of fibroblast growth factor 23 (FGF23) in hypophosphatemic patients: proposal of diagnostic criteria using FGF23 measurement

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Bone 2008;42:1235–1239

Background: Fibroblast growth factor 23 (FGF23) is an important regulator of phosphate homeostasis. It is critical for the development of hypophosphatemic diseases such as tumor-induced osteomalacia (TIO) and X-linked hypophosphatemic rickets/osteomalacia (XLH). However, any clinical usefulness of measurement of FGF23 has not been established. The objective of this study was to examine the importance of FGF23 measurement in the diagnosis of hypophosphatemic diseases.

Methods: Biochemical parameters concerning phosphate homeostasis were analyzed in a cross-sectional study. The study subjects included 32 patients with TIO, 28 with XLH and 16 patients with hypophosphatemia secondary to other causes including vitamin D deficiency, Fanconi's syndrome and Cushing's syndrome.

Results: FGF23 was above the upper limit of the reference range in most patients with TIO and XLH, irrespective of their medical treatment. The lowest FGF23 level in these patients was 38.0 pg/ml. FGF23 in hypophosphatemic patients with other causes was undetectable (<3 pg/ml) in 12 patients and the highest FGF23 in this group was 23.9 pg/ml. A relationship between the phosphate and FGF23 levels indicated that TIO and XLH are diseases with high FGF23 and hypophosphatemia judged by age-dependent reference ranges for serum phosphate.

Conclusion(s): Determination of FGF23 serum levels is useful for the differential diagnosis of hypophosphatemic diseases caused by excess actions of FGF23 like TIO, XLH, and other etiologies.

Over the last years, considerable progress has been made in our understanding of the molecular basis of the different forms of hypophosphatemic rickets [1]. The common denominator has been the hormone FGF23, a protein produced by osteoblasts and osteocytes with phosphaturic action [3, 4]. The enzyme PHEX inactivates FGF23 by cleavage. In X-linked hypophosphatemic rickets (XLH) the PHEX gene is mutated (inactive), whereas in the autosomal dominant form, FGF23 is mutated in the PHEX cleavage site. In tumor-induced osteomalacia (TIO), there is overproduction of FGF23 from the tumor cells. The loss of DMP1 gene function is the cause of autosomal recessive hypophosphatemic rickets and many of these patients have elevated FGF23 serum levels. In addition, there are other forms of hypophosphatemic rickets, some of which are secondary to other causes. In these cases, any role for FGF23 is unknown. Assays have been developed for the determination of FGF23 in human serum but the clinical usefulness of these assays has not yet been well established. These authors have used an assay, which is well characterized, measuring intact FGF23. The serum levels of FGF23 were determined in patients with TIO, XLH at various ages and in 16 patients with other causes of hypophosphatemia. As it has been previously described [7, 8], patients with XLH and TIO have high FGF23 serum levels. In addition, the authors found that patients with TIO had higher FGF23 levels than patients with XLH. In 12 out of 16 patients with other causes of hypophosphatemia, FGF23 serum values were undetectable and the rest of them had <30 pg/ml with the upper normal limit at 50 pg/ml. The authors propose that determination of FGF23 could be useful in discriminating hypophosphatemia due to excess FGF23 action (XLH, TIO) from other etiologies. This could be useful in pediatric patients with hypophosphatemia and no positive family history of hypophosphatemic rickets to differentiate as an example between XLH and hypophosphatemic rickets secondary to Fanconi syndrome. To clarify the clinical usefulness of serum FGF23 determinations in pediatric patients, additional studies including large numbers of patients with various types of hypophosphatemia are needed.

Important for clinical practice Normative data for bone health in children

The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race

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J Clin Endocrinol Metab 2007;92:2087–2099

Background: Low bone mass is associated with an increased risk of fractures. Furthermore, bone mineral accrual during childhood is a critical determinant of osteoporosis later in life. Several chronic medical conditions, medications, and lifestyle factors affect bone mineral accrual and thus appropriate reference values are essential for identification of children with bone deficits. The authors aimed at establishing reference curves for bone mineral content (BMC) and density (BMD) in children.

Methods: Study participants included 1,554 healthy children (761 male, 793 female), 6–16 years of age, of all ethnicities. They were enrolled in the Bone Mineral Density in Childhood Study, which is an ongoing longitudinal study. Measurements were obtained annually at five clinical centers in the USA. Scans of the whole body, lumbar spine, hip, and forearm were obtained using dual-energy x-ray absorptiometry

(DXA). Percentile curves based on three annual measurements were generated using the LMS statistical procedure.

Results: At enrolment, the height, weight and body mass index Z-scores were significantly greater than zero, indicating that the children were tall and heavy. At enrolment, 681 children were prepubertal and 873 were pubertal. Of the 1,554 children enrolled, 96 and 93% returned for 1- and 2-year measurements, respectively. Information for BMC of the whole body and lumbar spine and BMD of the whole body, lumbar spine, total hip, femoral neck, and forearm were given for specific percentiles by sex, age, and race (Black vs. non-Black). BMC and BMD were higher for Blacks at all skeletal sites ($p < 0.0001$). BMC and BMD increased with age, and a plateau was not evident by age 16 (girls) or age 17 (boys). The variation in BMC and BMD also increased with age.

Conclusion(s): There has long existed a clear need for pediatric BMC and BMD reference data. Here the age-, race-, and sex-specific reference curves are provided and they can be used to help identify children with impaired bone mineral accrual. In addition, the reference curves can be used in monitoring changes in response to chronic diseases or therapies.

DXA (previously DEXA) is a means of measuring BMD. Special considerations are involved in the use of DXA to assess bone mass in children. Specifically, comparing the BMD of children to the reference data of adults (T-score) will underestimate the BMD of children. To avoid an overestimation of bone mineral deficits, BMD scores are commonly compared to reference data for the same gender and age (by calculating a Z-score). But size has not been adjusted for. Appropriate pediatric BMC and BMD reference data has been lacking for many years. In order to respond to this need, Kalkwarf et al. have performed thousands of DXA measurements in five clinical centers in the USA and collected reference values for both BMC and BMD according to age, sex, and race. This is the first report from a large multicentered sample of children describing ethnic-specific BMC and BMD reference values. The data is based on the use of a standardized protocol. Clinical practitioners dealing with bone health in children should read this paper carefully, since it contains the reference values to determine a child's percentile rank for BMC and BMD – similar to the use of growth charts for height and weight. One drawback is the fact that this is another BMD reference with no reference to height, weight and puberty, all important determinants of pre-adult BMD. Delayed growth and maturation may complicate the interpretation of DXA findings in chronically ill patients. In addition, although children with low BMD may have an increased risk of fractures, a pediatric 'fracture threshold' has not yet been established.

Clinical trials

New treatment of hypophosphatemic rickets

Calcimimetics as an adjuvant treatment for familial hypophosphatemic rickets

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Clin J Am Soc Nephrol 2008;3:658–664

Background: Secondary hyperparathyroidism and nephrocalcinosis are the most severe complications in X-linked hypophosphatemia (XLH) treated with phosphate and calcitriol. In addition, vitamin D and phosphate stimulate FGF23 production, which is the pathogenic factor causing XLH. In patients with XLH it was investigated: (1) whether treatment with the calcimimetic agent, cinacalcet, will block the rise in parathyroid hormone (PTH) caused by phosphate administration, and (2) whether treatment with oral phosphate and calcitriol increases FGF23 levels.

Methods: Eight patients with XLH received a single oral dose of phosphate, followed the next day by combined treatment with phosphate and cinacalcet. Serum ionized calcium (Ca), phosphate, creatinine, intact PTH, 1,25(OH)₂D, FGF23, and tubular threshold for phosphate/glomerular filtration rate (TP/GFR) were assessed in response to short-term treatment with phosphate and cinacalcet and compared with long-term administration of phosphate and calcitriol.

Results: Oral phosphate load increased serum phosphate, decreased ionized calcium, and increased PTH. 24 h later, FGF23 significantly increased and $1,25(\text{OH})_2\text{D}$ decreased. The concomitant administration of phosphate and cinacalcet resulted in further decrease in serum Ca^{2+} with suppression of PTH and greater increase in serum phosphate and TP/GFR. Chronic treatment with phosphate and calcitriol resulted in a smaller increment in serum phosphate and high serum FGF23.

Conclusion(s): Traditional therapy of XLH with phosphate and calcitriol increases further FGF23 and therefore has the potential to stimulate PTH. Short-term treatment with cinacalcet suppresses PTH, leading to increase in TP/GFR and serum phosphate. Further studies are needed to investigate whether cinacalcet may be a useful adjuvant in the treatment of XLH, in order to allow the use of lower doses of phosphate and calcitriol, and therefore diminish the incidence of hyperparathyroidism and nephrocalcinosis.

The standard therapy of hypophosphatemic rickets with phosphorus supplementation and calcitriol when its levels are low is effective in raising serum phosphorus, healing rickets and osteomalacia and improving linear growth (when compliance is good), but it is often complicated by the development of nephrocalcinosis and secondary hyperparathyroidism. Many times the management of those patients is difficult due to these complications and we are still far from the optimum treatment. Calcimimetics are compounds that allosterically modulate the calcium-sensing receptor (CaSR) in the chief cells of the parathyroid gland, thereby enhancing its sensitivity to circulating serum calcium concentrations and consequently decreasing PTH secretion. Cinacalcet is a calcimimetic, which has recently been approved for the treatment of secondary hyperparathyroidism in end-stage renal disease [9]. Theoretically, calcimimetics could be useful in the treatment of hypophosphatemic rickets by suppressing PTH, which in turn can increase serum phosphorus. This could allow the use of lower doses of phosphate supplementation and calcitriol, thus decreasing the risk for nephrocalcinosis and secondary hyperparathyroidism. Based on that assumption, the authors studied if acute treatment of XLH patients with cinacalcet can increase serum phosphorus without increasing PTH levels. The authors found that cinacalcet increased serum phosphorus more than phosphate supplementation alone. In addition, it suppressed PTH levels, decreased serum calcium and decreased phosphorus excretion by the kidneys. This acute cinacalcet treatment had no effect on FGF23 serum levels. The data are interesting and promising. The increase in serum phosphorus with concurrent suppression of PTH could be useful in the treatment of hypophosphatemic rickets. Cinacalcet treatment in these situations could allow us to use lower doses of phosphate supplementation and calcitriol in order to decrease the risk for nephrocalcinosis and secondary hyperparathyroidism. In spite of the considerable progress over the last years in our understanding of phosphorus homeostasis and underlying molecular defects in hypophosphatemic rickets, little has been changed in our problematic treatment. More clinical studies investigating the long-term efficacy and safety of calcimimetics as an adjuvant treatment in hypophosphatemic rickets are now needed.

New mechanisms The Klotho story continues

A translocation causing increased α -Klotho level results in hypophosphatemic rickets and hyperparathyroidism

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Proc Natl Acad Sci USA 2008;105:3455–3460

Background: Phosphate homeostasis is important for a variety of diverse physiologic processes including energy homeostasis, formation of lipid bilayers, and bone formation. Hypophosphatemia due to renal loss causes hypophosphatemic rickets, a disease characterized by bone defects due to affected bone mineralization. On the other hand, hyperphosphatemia, a major complication of renal failure, is accompanied by parathyroid hyperplasia, hyperparathyroidism, and osteodystrophy.

Results: The authors present a patient with hypophosphatemic rickets and hyperparathyroidism due to parathyroid hyperplasia with distinctive facial features making the patient distinguishable from a case of classical X-linked hypophosphatemic rickets. The patient was found to have a de novo translocation with a breakpoint adjacent to α -Klotho, which encodes a β -glucuronidase, and is implicated in aging and regulation of FGF23 signaling. Plasma α -Klotho levels and β -glucuronidase activity were markedly increased in the affected patient. Unexpectedly, the level of circulating FGF23 was also markedly increased.

Conclusion(s): The authors conclude that the elevated level of α -Klotho mimics the aspects of the normal response to hyperphosphatemia and implicates that α -Klotho plays a role in the selective regulation of phosphate levels and parathyroid mass and function. They also speculate that their findings could have implications for the pathogenesis and treatment of renal osteodystrophy in patients with kidney failure.

A homozygous missense mutation in human Klotho causes severe tumoral calcinosis

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J Clin Invest 2007;117:2684–2691

Background: Familial tumoral calcinosis is characterized by ectopic calcifications with or without hyperphosphatemia. It is caused by inactivating mutations in FGF23 or UDP-N-acetyl- α -D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-3 (GALNT3).

Results: The authors report in this paper a 13-year-old girl who presented with severe tumoral calcinosis with dural and carotid artery calcifications and a homozygous missense mutation (H193R) in the *Klotho* gene. The patient had marked hyperphosphatemia and hypercalcemia as well as elevated serum levels of parathyroid hormone and FGF23. In vitro studies showed that, compared with wild-type *Klotho*, expression and secretion of H193R Klotho were markedly reduced, resulting in diminished ability of FGF23 to signal via its cognate FGF receptors.

Conclusion(s): It was concluded that loss-of-function mutations in the human *Klotho* gene impair FGF23 bioactivity, underscoring the essential role of *Klotho* in FGF23-mediated phosphate and vitamin D homeostasis in humans.

Already in the *Yearbook 2007* [10], we discussed the role of the new *Klotho* gene in knockout mice [11, 12]. This year, we come back to this exciting gene as de novo mutations have now been discovered in humans. Just to remind you, *Klotho* is an antiaging gene product, which potentiates the actions of FGF23. Knockout mice for *Klotho* and *FGF23* have similar premature aging phenotypes. In addition, *Klotho* knockout mice have hyperphosphatemia, enhanced renal phosphate reabsorption and growth retardation, just like FGF23-deficient mice. The role of Klotho has so far not been clarified in humans. These two papers very nicely describe the consequences of human Klotho excess and deficiency, respectively. When putting these clinical cases together with previous animal data, the role of Klotho has been clarified. The first paper describes a patient with hypophosphatemia due to renal loss, hyperparathyroidism, radiographic findings of rickets, and distinct facial features, which are not common in hypophosphatemic rickets. Despite the appropriate treatment, she developed early secondary hyperparathyroidism demanding parathyroid surgery, leaving half a gland. At the age of 19 years, hyperparathyroidism recurred and 75% of the remnant parathyroid gland had to be removed. Despite this, at the age of 23 years she again developed hyperparathyroidism. Sequencing analysis of the *PheX*, *FGF23*, *DMP1*, and *FGFR1* genes could not detect any mutations. However, the karyotype revealed a balanced translocation with a breakpoint adjacent to the α -Klotho gene causing an increased serum level of the Klotho protein. In addition, an elevated serum level of FGF23 was detected, a finding which is in line with in vitro and animal studies showing that Klotho enhances FGF23 signaling. The fact that mice with hypophosphatemia due to a mutated *PHEX* gene have low serum levels of Klotho speaks against the possibility that the observed high levels of Klotho in this patient are simply a secondary consequence of the hypophosphatemia. The second paper describes a patient with tumoral calcinosis, hyperphosphatemia, hypercalcemia and increased serum levels of FGF23 and PTH, who was found to have a homozygous missense mutation in the *Klotho* gene. Despite biochemical similarities, such as high circulating levels of FGF23 and hyperphosphatemia,

Fig. 2. Serum-derived phosphorus (P) and 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2$ Vit D) stimulate FGF23 production in bone. FGF23 requires Klotho for the activation of the FGFR receptor (FGFR) in the kidney which leads to suppression of P reabsorption and $1,25(\text{OH})_2$ Vit D synthesis. FGF23 is also regulated by DMP1 and PHEX since their deficiencies increase FGF23, but the exact mechanisms are not known. Finally, GALNT3 is required for the glycosylation and stabilization of FGF23. In humans, FGF23 overproduction by tumors is the cause of tumor-induced osteomalacia. Autosomal dominant hypophosphatemic rickets is caused by FGF23 gain-of-function mutations, X-linked hypophosphatemic rickets by mutations in the PHEX gene, whereas DMP1 mutations cause autosomal recessive hypophosphatemic rickets.

there are several biochemical differences between *Klotho*-deficient mice and this patient. First, although *Klotho*-deficient mice have increased urinary calcium excretion, the patient did not have hypercalciuria. Secondly, *Klotho*-deficient mice have abnormal regulation of insulin/IGF-I signaling, resulting in hypoglycemia and increased insulin sensitivity. In contrast, the patient had normal fasting glucose and insulin levels. Third, this patient has a high serum level of PTH, in contrast to *Klotho*-deficient mice and patients with tumoral calcinosis associated with *FGF23* or *GALNT3* mutations that have low or normal PTH levels. Fourth, premature aging was not reported in the patient as has been demonstrated in *Klotho*-deficient mice. As summarized in figure 2, these two papers show that Klotho is important for enhancing FGF23 signaling, but more work has to be done to further elucidate this complex, but very interesting, pathway.

New mechanisms Critical hedgehog pathway in bone

Indian hedgehog produced by postnatal chondrocytes is essential for maintaining a growth plate and trabecular bone

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Proc Natl Acad Sci USA 2007;104:6382–6387

Background: Indian hedgehog (Ihh) is well known to be essential for normal prenatal chondrocyte and osteoblast proliferation/differentiation. The early lethality of Ihh-ablated mutant mice has so far prevented further analysis of its role in postnatal bone growth and development.

Methods: To resolve this issue, tamoxifen-inducible conditional knockout mice were generated where the Ihh gene was successfully ablated from postnatal chondrocytes in a temporal/spatial-specific manner.

Results: Postnatal deletion of Ihh resulted in loss of columnar structure, premature vascular invasion, and formation of ectopic hypertrophic chondrocytes in the growth plate. In addition, the articular surfaces were found to be destructed and the growth plates to be prematurely fused which resulted in

dwarfism in mutant mice. These mutant mice also exhibited continuous loss of trabecular bone over time.

Conclusion(s): The data clearly demonstrate that postnatal chondrocyte-derived *Ihh* is essential for maintaining the growth plate and articular surface and is required for sustaining trabecular bone and skeletal growth.

Transient inhibition of the hedgehog pathway in young mice causes permanent defects in bone structure

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Cancer Cell 2008;13:249–260

Background: The hedgehog (Hh) pathway is known to be critical not only for normal bone development but also to play an important role in tumorigenesis.

Methods: Gli-luciferase transgenic mice were generated to evaluate the effects of a small molecule antagonist of Smo (HhAntag) on postnatal bone development. Whole animal functional imaging was applied.

Results: HhAntag rapidly reduced systemic luciferase activity in 10- to 14-day-old mice following oral dosing. Although pathway activity was restored 2 days after drug removal, brief inhibition caused permanent defects in bone growth. HhAntag inhibited proliferation and stimulated differentiation of the growth plate chondrocytes. As a consequence, the hypertrophic layer was dramatically expanded. After drug removal, osteoblasts invaded the growth plate, mineralization occurred, and premature fusion of the growth plate occurred which resulted in permanent disruption of the epiphyses.

Conclusion(s): Transient inhibition of the hedgehog pathway rapidly promotes chondrocyte differentiation which leads to an expansion of the hypertrophic zone and breakdown of the columnar organization. The aberrant structures formed could not be removed by bone remodeling, so the defects were irreversible.

Hedgehog signals are transduced through smoothened (Smo), a putative G-protein-coupled seven-transmembrane domain protein, in concert with PTHrP [13]. In the absence of hedgehog proteins, Smo is repressed by the *Ihh* target gene *Ptch*, another cell surface receptor for hedgehog. The majority of *Ihh*-null embryos die during early development, and it has therefore not been possible to clarify the actions of hedgehog proteins during postnatal bone development by knockouts. To resolve this issue, the authors of the first paper generated tamoxifen-inducible conditional knockout mice, where the *Ihh*-gene was selectively targeted in cartilage and bone during postnatal development. The data clearly demonstrate that *Ihh* expression in chondrocytes is critical for the maintenance of normal postnatal growth and growth plate architecture. Therefore, it is not surprising that the authors of the second paper found that transient inhibition of the hedgehog pathway with a small molecule antagonist of Smo (HhAntag) caused irreversible destruction of the growth plate and permanent growth arrest in treated mice. The data suggest that short-term exposure to signal transduction inhibitors during postnatal development can have long-term consequences raising concerns about the potential side effects associated with cancer therapies targeting the hedgehog pathway in children. An important lesson is the need for functioning communication between cartilage and bone, even in postnatal life, to maintain postnatal growth plate structures and for preserving trabecular bones. Nevertheless, although the impact of brief suppression of the hedgehog pathway on bone development in mice is quite striking, this should not preclude attempts to develop highly promising anticancer treatment based on targeting of the hedgehog pathway.

Multiple genetic loci for bone mineral density and fractures

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N Engl J Med 2008;358:2355–2365

Background: Osteoporosis is a common skeletal disorder characterized by compromised bone strength and increased risk of fractures. Bone mineral density (BMD) is the single best predictor of osteoporotic fractures, and it is also influenced by environmental and medical factors. The aim of this study was to identify sequence variants associated with bone mineral density and fracture.

Methods: The authors performed a quantitative trait analysis of data from 5,861 Icelandic subjects (the discovery set), testing for an association between 301,019 single-nucleotide polymorphisms (SNPs) and bone mineral density of the hip and lumbar spine. They then tested for an association between 74 SNPs (most of which were implicated in the discovery set) at 32 loci in replication sets of Icelandic, Danish, and Australian subjects (4,165, 2,269, and 1,491 subjects, respectively).

Results: Sequence variants in five genomic regions were significantly associated with BMD in the discovery set and were confirmed in the replication sets (combined *p* values, 1.2×10^{-7} to 2.0×10^{-21}). Three regions were close to or within genes previously shown to be important to the biologic characteristics of bone. The genes were receptor activator of nuclear factor- κ B ligand (RANKL) (chromosomal location, 13q14), osteoprotegerin (OPG) (8q24), and estrogen receptor 1 (ESR1) (6q25). The two other regions that were associated with BMD were close to the zinc finger and BTB domain containing 40 gene (ZBTB40) (1p36) and the major histocompatibility complex region (6p21). Other loci associated with osteoporotic fractures were at 1p36, 8q24, and 6p21, as were loci at 18q21, close to the receptor activator of the nuclear factor- κ B gene (RANK), and loci at 2p16 and 11p11.

Conclusion(s): The authors discovered common sequence variants that were consistently associated with BMD and with low-trauma fractures in three populations of European descent. These variants provide insight into the biochemical pathways underlying osteoporosis even though they cannot be used alone in the clinical prediction of an individual's fracture risk.

Besides BMD, many other factors, such as age, menopausal stage, smoking, physical activity, diet, coexisting diseases and pharmacological treatments, influence the risk of osteoporosis. One of the most clinically important risk factors is a family history of the disorder. Similar to other complex disorders, alterations in many different genes contribute to susceptibility. Thus, researchers have been seeking for relevant genes by linkage analyses and association studies. However, these methods have often been problematic, since they cannot detect modest changes that are often seen in complex diseases. With improved genotyping technologies for single-nucleotide polymorphisms (SNPs), it has become possible to search genome-wide for risk factors in large cohorts of individuals. The study by Styrkarsdottir et al. is the first large and comprehensive genome-wide association study of the relevant genes associated with BMD and low-trauma fractures. They identified five loci for which there is strong evidence of association with BMD: three previously identified genes (ESR1, OPG gene and RANKL gene), as well as two new loci (1p36 and 6p21). The variants with the strongest association for BMD were tested for association with a fracture in an independent cohort, and there proved to be modest but significant evidence for association for the two new loci, as well as for the OPG gene. The value of these findings in the prediction of fracture risk is limited but they provide new elements in the osteoporosis network. Validated genetic associations provide direct evidence of the causality of a particular gene or a pathway in a disease progress, and in the future they might be useful in stratifying patients according to the risk and for different treatment regimens.

New hormones

Novel roles for CNP during chondrogenesis

C-type natriuretic peptide regulates cellular condensation and glycosaminoglycan synthesis during chondrogenesis

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Endocrinology 2007;148:5030–5041

Background: C-type natriuretic peptide (CNP) was recently identified as a key anabolic regulator of endochondral bone growth. However, the cellular and molecular mechanisms involved have not been well characterized.

Methods: The effects of CNP were investigated in micromass cultures of mouse embryonic limb bud cells.

Results: CNP increased the number of chondrogenic condensations an effect which was accompanied by increased expression of the cell adhesion molecule N-cadherin. In addition, CNP enhanced the synthesis of glycosaminoglycan, an effect which was accompanied by increased expression of enzymes involved in chondroitin sulfate synthesis.

Conclusion(s): The authors demonstrate a novel role of CNP in promoting chondrogenesis by stimulating expression of molecules involved in cell adhesion molecules and glycosaminoglycan synthesis.

The list of growth plate regulators grows steadily. As was reviewed in the *Yearbook* 2007 [10], C-type natriuretic peptide (CNP), a member of the natriuretic peptide family, has been documented to regulate endochondral ossification through the promotion of chondrocyte proliferation and hypertrophy in the growth plate [14]. In the present paper, Woods et al. demonstrate a role for CNP to modulate chondrocyte differentiation in the early stages of chondrogenesis, both through the regulation of cellular condensations and through the synthesis of glycosaminoglycans. The authors show that as cells differentiate into chondrocytes, they dramatically increase expression of the CNP receptor *Npr2*, providing a mechanism for CNP signaling. Compared with the strong increase in *Npr2* levels, the biological effects of CNP treatment appear somewhat modest. One potential explanation is that the simultaneous up-regulation of *Npr3*, encoding the decoy receptor, limits cellular responses to CNP. These data suggest that the biological activities of CNP in cartilage are tightly regulated which is in agreement with the recent observation that CNP induces *Npr3* expression at later stages of chondrocyte differentiation [15]. It is possible that these novel functions of CNP contribute both to growth retardation in response to loss of CNP signaling and to the potential therapeutic effects of exogenous CNP.

Reviews

We still need to fight against vitamin D deficiency

Vitamin D deficiency: a worldwide problem with health consequences

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Am J Clin Nutr 2008;87:1080S–1086S

Summary: The authors point out that the incidence of vitamin D deficiency has increased. They review the causes of vitamin D deficiency (mainly the lack of appreciation that sun exposure in moderation is essential), and they remind us that very few foods naturally contain vitamin D, and foods that are fortified with vitamin D are often inadequate to satisfy either a child's or an adult's vitamin D requirement. Vitamin D deficiency is not only a clinical entity in children where it causes rickets, but also in adults where it causes osteopenia, osteoporosis, and fractures. In addition, vitamin D deficiency has been associated with increased risk of common cancers, autoimmune diseases, hypertension, and infectious

diseases. A circulating level of 25-hydroxyvitamin D of >75 nmol/l, or 30 ng/ml, is required to maximize vitamin D's beneficial effects for health. In the absence of adequate sun exposure, at least 800–1,000 IU vitamin D₃/day may be needed to achieve this in children and adults. Vitamin D₂ may be equally effective for maintaining circulating concentrations of 25-hydroxyvitamin D when given in physiologic concentrations.

This is a well-written review on vitamin D deficiency, which is worth reading. It not only discusses the causes and diagnosis of vitamin D deficiency but also the serum levels of vitamin D that are needed throughout life. In addition, the review gives a historical perspective on vitamin D deficiency. An interesting part of the article is the discussion on non-skeletal consequences of vitamin D deficiency including the association with increased risks of common cancers, autoimmune diseases, hypertension, and infectious diseases. In addition, the prevention and treatment of vitamin D deficiency is discussed and the authors stress that foods fortified with vitamin D do not by themselves cover the basal vitamin D requirements. The authors also point out the need for a re-evaluation of the recommended daily intake of vitamin D which may need to be increased in both children and adults. As was recently reported by a collaborative ESPE effort, the unique environment and genetic predisposition may also have to be accounted for in the design of preventive measures, rather than using a standard recommended dietary intake for calcium and vitamin D [16].

Reviews

A unifying new somatomedin hypothesis

The somatomedin hypothesis 2007: 50 years later

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J Clin Endocrinol Metab 2007;92:4529–4535

Summary: Newer experimental evidence has led to several modifications of the original somatomedin hypothesis, but none of the proposed modifications accounts for all of the integrated actions of GH and IGF-I. Kaplan and Cohen now present a modification of the existing hypothesis that takes into account all the actions of the GH-IGF system. The modification is based on experimental evidence published since the original hypothesis was developed. The modification is based on an integration of the results of published experimental evidence regarding the actions of GH and the IGF complex. The authors present a new augmentative/counteractive modification of the hypothesis. They propose that the actions of the GH-IGF system provide a distinct evolutionary advantage to the organism by augmenting the anabolic actions of GH while countering its potentially harmful effects of hyperglycemia and depletion of lipid stores.

The initial somatomedin hypothesis was based primarily on a series of elegant experiments conducted half a century ago. In 1957, Salmon and Daughaday [17] showed that incorporation of radioactive inorganic sulfate into acid mucopolysaccharides of rat cartilages could be stimulated by addition of serum from hypophysectomized rats that had been injected with GH *in vivo*. Addition of GH to the medium in which the cartilages were being incubated, however, did not result in enhanced incorporation of the radioactive precursor. Today, it is evident that IGF-I cannot be the sole mediator of GH action because IGF-I repletion does not restore all the deficits found in GH insensitivity. It is also evident that GH has direct effects *in vivo* that are independent of IGF-I, many of which are exerted through the local production of IGFs rather than under the influence of a factor in the circulation. The authors cite evidence that the anabolic actions of IGF-I are augmentative to those of GH, whereas the effects on lipid and carbohydrate metabolism run counter to the established actions of GH. Based on this, the authors formulated their modified somatomedin hypothesis which proposes that the IGFs, rather than being effectors of GH action, are augmentative hormones that amplify the anabolic actions of GH while countering its potentially deleterious effects. Although this may not be the complete story explaining the interactions of GH and IGF-I, it is an attractive new unifying hypothesis.

Expression profiling of dexamethasone-treated primary chondrocytes identifies targets of glucocorticoid signalling in endochondral bone development

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BMC Genomics 2007;8:205

Background: Patients taking glucocorticoids often suffer from skeletal side effects including growth retardation in children and adolescents, and decreased bone quality in adults. Glucocorticoids have been implicated in the regulation of chondrogenesis and osteoblast differentiation, as well as maintaining homeostasis in cartilage and bone. Some targets of glucocorticoid regulation in chondrogenesis are known, but the global effects of pharmacological glucocorticoid doses on chondrocyte gene expression have not been comprehensively evaluated.

Methods: Primary limb bud mesenchyme micromass cultures were exposed to dexamethasone followed by a microarray screen applying the Affymetrix platform.

Results: Dexamethasone was found to act in a gene class-specific manner promoting the expression of extracellular matrix and metabolic transcripts necessary for maintaining the chondrocyte phenotype, while simultaneously down-regulating cytokines and growth factors which stimulate the cartilage to bone transition. Comparing dexamethasone-induced gene expression data to developmental changes in gene expression in micromass cultures revealed an additional layer of complexity in which dexamethasone maintains the expression of certain chondrocyte marker genes, while inhibiting factors that promote vascularization and ultimately ossification of the cartilaginous template.

Conclusion(s): The data provide insight into the mechanisms and major molecular classes functioning downstream of dexamethasone in primary chondrocytes.

Patients taking glucocorticoids often suffer from skeletal side effects including growth retardation in children and adolescents, and decreased bone quality in adults. The authors of this paper aimed to identify new targets to prevent the undesired side effects of glucocorticoid treatment on chondrocytes. They very elegantly detail the downstream transcriptional impact of pharmacological glucocorticoid exposure on developing chondrocytes. When comparing the data with microarray studies of glucocorticoid treatment in other cell types, it is evident that the majority of the effects are tissue-specific. This study provides novel insights into the effects of pharmacological glucocorticoid exposure on chondrocyte gene transcription and establishes the foundation for subsequent functional studies. The ultimate objective must of course be to translate these findings into safer and more efficacious treatment with glucocorticoids. As reviewed in *Yearbook 2007* [10], selective glucocorticoid receptor modulators may be the way to go as they allow the selective targeting of glucocorticoid regulated genes.

References

1. Tiosano D: Bone and Mineral Metabolism; in *Yearbook of Pediatric Endocrinology* 2004. Amsterdam, Elsevier, 2004, pp 47–64.
2. Ferron M, Hinoi E, Karsenty G, Ducy P: Osteocalcin differentially regulates β -cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. *Proc Natl Acad Sci USA* 2008;105:5266–5270.
3. Liu S, Quarles LD: How fibroblast growth factor 23 works. *J Am Soc Nephrol* 2007;18:1637–1647.
4. Yoshiko Y, Wang H, Minamizaki T, Ijuin C, Yamamoto R, Suemune S, et al: Mineralized tissue cells are a principal source of FGF23. *Bone* 2007;40:1565–1573.
5. Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, et al: Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* 2001;97:1227–1231.
6. Le Blanc K, Gotherstrom C, Ringden O, Hassan M, McMahon R, Horwitz E, et al: Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. *Transplantation* 2005;79:1607–1614.
7. Imel EA, Peacock M, Pitukcheewanont P, Heller HJ, Ward LM, Shulman D, et al: Sensitivity of fibroblast growth factor 23 measurements in tumor-induced osteomalacia. *J Clin Endocrinol Metab* 2006;91:2055–2061.

8. Yamazaki Y, Okazaki R, Shibata M, Hasegawa Y, Satoh K, Tajima T, et al: Increased circulatory level of biologically active full-length FGF-23 in patients with hypophosphatemic rickets/osteomalacia. *J Clin Endocrinol Metab* 2002;87:4957–4960.
9. Block GA, Martin KJ, de Francisco AL, Turner SA, Avram MM, Suranyi MG, et al: Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. *N Engl J Med* 2004;350:1516–1525.
10. Chrysis D, Heino T, Sävendahl L: Growth Plate, Bone and Calcium; in *Yearbook of Pediatric Endocrinology* 2007. Basel, Karger, 2007, pp 53–70.
11. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al: Mutation of the mouse *Klotho* gene leads to a syndrome resembling ageing. *Nature* 1997;390:45–51.
12. Razzaque MS, Lanske B: Hypervitaminosis D and premature aging: lessons learned from *Fgf23* and *Klotho* mutant mice. *Trends Mol Med* 2006;12:298–305.
13. Chagin A, Dahlgren J, Sävendahl L: Growth Plate-Cellular Growth; in *Yearbook of Pediatric Endocrinology* 2005. Basel, Karger, 2005, pp 47–62.
14. Olney RC: C-type natriuretic peptide in growth: a new paradigm. *Growth Horm IGF Res* 2006;16(suppl A):S6–S14.
15. Agoston H, Khan S, James CG, Gillespie JR, Serra R, Stanton LA, et al: C-type natriuretic peptide regulates endochondral bone growth through p38 MAP kinase-dependent and -independent pathways. *BMC Dev Biol* 2007;7:18.
16. Baroncelli GI, Bereket A, El Kholy M, Audi L, Cesur Y, Ozkan B, et al: Rickets in the Middle East: role of environment and genetic predisposition. *J Clin Endocrinol Metab* 2008;93:1743–1750.
17. Salmon WD Jr, Daughaday WH: A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *J Lab Clin Med* 1957;49:825–836.