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A SUPPLEMENT TO PEDIATRICS

Effects of Pharmacologic Agents on Bone in Childhood

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ON APRIL 14, 2005, a workshop on the skeletal effects of pharmacologic agents in children was held at the National Institutes of Health. Jointly sponsored by the American Society for Bone and Mineral Research (ASBMR) and the National Institute of Child Health and Human Development, the meeting was organized in response to suggestions from the Pediatric Bone Initiative of the ASBMR. Previous discussions at the 2003 ASBMR symposium on the state of pediatric bone research led to the conclusion that there was a lack of information on the effects of Food and Drug Administration–approved therapeutic agents on bone in children. Advances in pediatric medicine have produced a new population of children who are able to live into adulthood with chronic illnesses and were previously thought to have a poor prognosis for survival beyond their childhood years. Medications and direct effects of illness may compromise normal bone mineralization, leading to skeletal deformities and osteopenia at a relatively young age. Several topics discussed at the 2003 ASBMR symposium were further examined and developed in the recent National Institutes of Health workshop, such as the limitations of currently available pediatric bone density–reference data, the challenges of measuring bone mass and strength in children, clinical trial design, chronic drug effects on growing bone, and therapeutic agents for pediatric bone disorders. The following is a summary of all the presentations from the National Institutes of Health workshop on pediatric bone. Detailed summaries of selected talks are provided as well.

The first session provided basic background on normal bone accrual in children, the regulation of skeletal growth, and the effects of calcium, phosphorus, and vitamin D on growing bone.

Dr Jeffrey Baron and his co-workers reviewed the role of the growth plate in linear skeletal growth and work from his own laboratory to elucidate mechanisms of growth at the growth plate. Addressing specifically the role of the resting zone of the growth plate in longitudinal growth, he presented evidence that the resting zone contains chondrocyte stem-like cells that can regenerate the entire growth plate in the absence of the other 2 zones, the proliferative and hypertrophic. He then discussed growth-plate senescence and, with the use of rabbit and rat models, hypothesized that this phenomenon is a result of the finite capacity of chondrocytes to proliferate. Dr Baron proposed that “catch-up” growth, which occurs after recovery from a specific condition that inhibits growth, is explained by changes in the tempo of growth-plate senescence. In this model, inhibition of linear growth slows growth-plate senescence. When the inhibiting condition is resolved, the growth-plate chondrocytes have more of their proliferative capacity remaining and, thus, catch-up growth occurs. Conversely, estrogen accelerates growth-plate senescence, which leads to earlier “exhaustion” of...
chondrocyte proliferative capacity and epiphyseal fusion.

Dr Baron then went on to challenge the widely held belief that bone acquisition in childhood is like a “bank account” (i.e., the belief that perturbations that lead to a transient decrease in bone acquisition during childhood will result in lower peak bone density). Instead, he hypothesized that periosteal new bone formation and endosteal bone resorption, which create a wider bone, would displace inadequately formed bone from a younger age. Moreover, Dr Baron then challenged the assumption that childhood and adolescent bone mass predicts adult bone mass. Evidence comes from work in his laboratory on a rabbit model in which dexamethasone-induced inhibition of bone accrual reversed when the dexamethasone treatment was stopped. At the metaphysis, this reversal was caused by the replacement of osteoporotic bone by healthy bone at the growth plate. At the periosteal surface, when the dexamethasone was stopped, the decrease in periosteal bone formation was halted and formation actually increased above normal. The mechanism for this catch-up growth at the periosteal surface is likely not the same as at the growth plate and may, instead, be a result of changes in mechanosensing. In humans, supportive evidence comes from the finding that the increase in bone density seen in children who received calcium supplements over 3 years disappears after treatment is stopped.

Dr Frank Rauch then spoke on the mechanisms behind the increase at the trabecular and periosteal surfaces that occur during childhood. At the trabecular surfaces, he provided evidence that changes in neither material density nor trabecular number account for the increase in trabecular bone mineral density (BMD) during puberty. Instead, studies performed in his laboratory have demonstrated that the increase in trabecular BMD during growth are caused by an increase in trabecular thickness and that this increase is caused by “remodeling with a positive balance,” which is a rather slow process (each remodeling cycle takes 9–10 months).

Next, Dr Rauch addressed the issue of bone growth in width, a poorly understood process that is just as crucial as bone growth in length. When the bone grows longer, the width must increase concomitantly to preserve bone strength. Periosteal bone formation in children differs from adults, not only because this process is more active in children but also because it is continuous (modeling), whereas in adults the process is cyclic (remodeling). Periosteal apposition is rapid early in life, slows with age, and is variable between bones, depending on the mechanical stimuli. Although systemic factors such as hormones are important in regulating periosteal bone growth, given that the growth is so site/bone specific, local factors such as mechanical load must play a predominant role.

In the last talk of this session, Dr Marie Demay addressed the effects of mineral homeostasis and vitamin D on growing bone and presented data from her laboratory on a vitamin D receptor (VDR)-knockout mouse model. This is the same defect seen in humans with hereditary vitamin D-resistant rickets. These mice developed rickets, osteomalacia, hypocalcemia, secondary hyperparathyroidism, and hypophosphatemia. However, when placed on a diet high in calcium, phosphorus, and lactose, all of the abnormalities were prevented, including the rickets and osteomalacia, which demonstrated that it is the secondary effects of the VDR on mineral metabolism, not the VDR itself, that are important for bone mineralization. Additional studies demonstrated that the abnormal mineral ion homeostasis led to expansion of the growth plate by impairing apoptosis of the late hypertrophic chondrocytes. Finally, Dr Demay demonstrated that phosphate mediates apoptosis of the hypertrophic chondrocytes via the mitochondrial apoptotic pathway. Overall, the studies presented by Dr Demay demonstrated that phosphate, not calcium, is crucial for apoptosis of the terminally differentiated chondrocytes and, thus, the mechanism by which VDR resistance leads to rickets.

The second session of the conference addressed the challenges of investigating bone health in the growing child. Dr Laura Bachrach outlined the barriers to recruitment and retention of subjects in clinical trials. An estimated 86% of US clinical trials in adults fail to complete cohort recruitment within the projected time frame, and attrition rates are typically 15% to 40%. Factors related to subjects, investigators, and protocols account for these failures. Potential subjects refuse to enroll or quit studies because of inconvenient or lengthy study visits, uncertainty about whether treatment will be provided, distrust of the research team, cultural or language barriers, or relocation. Minority group members and individuals with less education are less likely to participate; one review found that 70% of intervention studies had underenrollment of minority subjects. Most relevant to the meeting focus, parents are less likely to enroll young children. Factors that reduce investigator participation in clinical research include the competing roles of care provider and investigator, inadequate time, resources, or incentives for research, competing clinical trials, and unstable research support teams. Protocol-related variables that impede success include overly demanding visits, restrictive inclusion and exclusion criteria, and delays in the start of the trial. Negative input from the media and lengthy institutional review board processes also hamper successful recruitment.

Despite these barriers, Dr Bachrach identified examples of longitudinal observation studies of bone health in children and adolescents that had achieved notable success in recruitment and retention. Factors that contributed to these successful investigations included consistent and personable study staff, a robust source of...
potential subjects, support from communities, schools, and relevant professional colleagues, convenient and efficient study visits, adequate incentives, and ongoing contact such as birthday or holiday greetings between study personnel and subjects between visits.

Dr Richard Henderson addressed the challenges of identifying appropriate enrollment criteria and therapeutic end points for pediatric trials. The most clinically relevant goal is the reduction of fractures. However, the annual fracture rate reaches 5% to 10% only in children with severe disabilities or osteogenesis imperfecta and is <3% for those with other chronic illnesses, which makes it hard to use fracture prevention as the standard for therapeutic success. Power calculations must take into account the frequency of fractures in untreated children, the anticipated effect size of active treatment, and the inevitable attrition during the study. Because control subjects will also likely benefit from receiving optimal standard-of-care treatment, such as vitamin D and calcium, this must also be factored into the model. In a sample “best-case” scenario, assuming a baseline annual fracture rate of 10%, an 80% reduction of fractures with active treatment, a 10% decrease in fractures in control subjects, and yearly attrition of 5%, a cohort of 92 subjects is needed. In a less favorable scenario with a 5% annual fracture rate, 40% treatment efficacy, 20% effect of control therapy, and 20% attrition, a cohort of 3422 subjects is needed. Recruiting a pediatric cohort of this size would be formidable. Dr Henderson also raised important ethical questions concerning the risk/benefit ratio when testing pharmacologic agents in children. Is it appropriate to enroll a child who has not yet had a fracture? Conversely, is it ethical to withhold a drug that has been proven efficacious for adult patients with osteoporosis from children who have already fractured a bone? Are proxy measures of bone fragility, such as BMD, sufficiently robust to use in select children at highest risk for enrollment in a study? Answers to these questions will influence the pool of potential subjects for recruitment, the heterogeneity of the cohort, and whether findings can be generalized to other patient groups.

Dr Vicente Gilsanz discussed the use of bone densitometry as an alternative end point for pediatric bone-health research. In adults, the relationship between BMD and fracture risk is sufficiently robust that BMD can be used to diagnose osteoporosis, select patients for treatment, and monitor response to drug therapy. The value of bone densitometry for predicting fractures in children is more controversial.

Densitometry results are considerably more challenging to interpret in the growing child because of the changes in bone size and shape that were outlined in earlier talks. In addition, the correlation between BMD and fracture risk is not well established in children. Dual-energy x-ray absorptiometry (DXA), the most commonly used densitometry technique, provides a two-dimensional measurement of three-dimensional bones; therefore, bone size influences BMD results. For example, low BMD of a child may reflect his or her smaller body size, and longitudinal increases in BMD can reflect changes in bone size, bone density, or both. To distinguish between changes in bone size and mineral, bone mass can be evaluated by quantitative computed tomography (QCT). This technique measures volumetric BMD and is not influenced by bone size. As detailed in an article by Dr Gilsanz and his co-workers,10 QCT and DXA provide different information regarding the skeletal status of children. In a study with chronically ill and healthy youth, 19% were identified as having low spine bone mass for age (z score less than −2) using areal BMD measurements from DXA as compared with only 6% using volumetric BMD measurements from QCT.11 Smaller bone size accounted for the low bone mass in many of the children.

A critical question is whether DXA or QCT is a better surrogate measure of bone fragility in childhood. This issue remains unresolved because there are too few data linking QCT or DXA to fracture risk in children. The choice of an appropriate surrogate measure for fracture is a key area of controversy that must be resolved when planning bone-health–intervention studies in pediatric populations.

The third session focused on the use of bisphosphonates in children. Professor Graham Russell reviewed the current models of bisphosphonate action as antiresorptive agents.12 These drugs accumulate on the denuded surfaces of bone surrounding resorbing osteoclasts where they are taken up by the osteoclastic cells. Nitrogen-containing bisphosphonates interfere with biosynthesis of lipids attached to guanosine triphosphate–bound proteins, inactivating the proteins and inhibiting osteoclast motility and function. Non–nitrogen-containing bisphosphonates act by reversing reactions involved with protein synthesis, which results in accumulation of toxic products and leads to cell apoptosis. Bisphosphonates may also reduce bone resorption by interfering with glucocorticoid-induced apoptosis of bone cells; however, details of this activity require clarification. Different bisphosphonate compounds attach to bone surfaces with variable degrees of avidity. This strength of attachment of a given drug determines whether the compounds are longer or shorter acting. Bisphosphonates have been tested to only a limited extent in growing children. Many uncertainties persist regarding their use in pediatrics, including which bisphosphonate is preferred, in which dose it should be used, and for how long treatment should last. Little is known about the duration of the antiresorptive effect once bisphosphonates are discontinued in younger patients.

Addressing this latter issue, Dr Craig Langman (unpublished data, 2005) examined the duration of the
antiresorptive action of alendronate in a cohort of 46 children with fracturing osteoporosis. The cohort of 46 subjects had been treated for a median of 672 days (1.8 years) and was examined at a median of 410 days after cessation of bisphosphonate therapy. Only 1 of the 46 children studied had a decline in lumbar spine BMD z score between treatment termination and follow-up; 2 children had sustained an interval fracture. Urinary N-telopeptide of type I collagen, a marker of bone resorption activity, remained suppressed. These data suggest that alendronate therapy sustains antiresorptive activity for at least 1 year after termination.

Dr Francis Glorieux reviewed the experience that he and his co-workers have had with bisphosphonate therapy in osteogenesis imperfecta (OI). OI is associated with a high rate of bone turnover, which, when combined with the decreased ambulation, deformities, and pain, leads to bone loss. For these reasons, bisphosphonates were initially tried as a treatment for OI. Pamidronate therapy in children with OI results in significantly decreased skeletal pain, increased sense of well-being, and increased volumetric BMD and bone volume compared with untreated patients with OI. Dr Glorieux’s group has performed controlled trials using alendronate in patients with OI. The results showed a significantly higher vertebral spine BMD z score in the treated versus untreated patients. The vertebral shape was improved in the patients in the treated group, and the gain in height of the vertebral body, coupled with the increase in BMD with treatment, results in an increased amount of bone in the vertebrae. In the patients in the alendronate-treated group there was a nonsignificant trend toward a decreased fracture incidence, significant improvement in gross motor function, and no negative effects on growth. Bone-biopsy results showed a dramatic increase in cortical thickness and a more modest increase in trabecular bone volume. There was a decrease in bone turnover, but remodeling continued. The stiffness typical of bone in patients with OI did not deteriorate with treatment. The adverse effects of pamidronate treatment included an acute-phase reaction with treatment and a delay in the healing of osteotomies, neither of which are well understood.

Dr Glorieux ended with advice for clinicians treating children with OI. He concluded that his studies of pamidronate and alendronate in OI favor pamidronate. He stressed that children with mild OI should not be treated with bisphosphonates other than in the clinical research setting. Also, regarding the issue of when to discontinue treatment, he pointed out that when bisphosphonates are discontinued, the bone reverts back to its original untreated state, which leaves the untreated bone prone to fracture.

Dr Michael Whyte addressed the issue of bisphosphonate toxicity and began with his sobering prediction that there may be a higher prevalence of adverse effects from bisphosphonate therapy in the near future, likely because of increased use of these medications. Excess bisphosphonate can lead to an osteopetrosis-like state. In a case of “bisphosphonate-induced osteopetrosis,” a boy presented to Dr Whyte and his co-workers after being treated with 4 times the dose of pamidronate recommended by Dr Glorieux’s group over a 3-year period. He had many of the signs, symptoms, and biochemical and bone-biopsy findings of genetic forms of osteopetrosis. Another patient, a 62-year-old woman with breast cancer who was treated for bone metastases with pamidronate and zoledronate for 7 years, had a very high BMD according to DXA and disturbances in bone remodeling. Other cases were presented that reiterated a disturbance in bone modeling and remodeling in children on bisphosphonates, which led to poor bone quality and an increased tendency to fracture. Bisphosphonate therapy has also been shown to lead to osteonecrosis of the jaw in adults, not children.

In an effort to deal with the complications of excessive bisphosphonate use, Dr Whyte’s group is proceeding with studies aimed at determining when someone may be at risk for excessive bisphosphonate effects by using tools such as bone biopsies, micro–computed tomography, and measurements of creatine kinase isoenzyme (CK-BB) and tartrate-resistant acid phosphatase. These studies may lead to a list of parameters that the clinician can follow when a patient receives bisphosphonates to determine when the patient should stop therapy because of risk of complication from excessive use.

Dr Joan Marini presented more data on trials of bisphosphonate therapy in children and mice with OI. In a placebo-controlled 2-year trial of olpadronate in the Netherlands, the treated group showed an increase in vertebral BMD and a decrease in the relative risk of long-bone fractures. No changes were seen in vertebral geometry, but the fact that approximately one third of the patients had type 1 OI may be explained by this negative finding. In the placebo-controlled alendronate trial discussed earlier by Dr Glorieux, the vertebral BMD increased, and there was a trend toward decreasing fracture rate in the treated group. Finally, the National Institute of Child Health and Human Development–sponsored trial of pamidronate in 18 children with types 3 and 4 OI showed increases in DXA z scores and improvement in vertebral geometry, midvertebral height, and vertebral area in the patients in the treated group during the first year but not the second year. There were no effects of treatment on fracture incidence, ambulation, muscle strength, or pain. Dr Marini concluded from these studies that bisphosphonates have a much more beneficial effect on the spine than on the long bones, and that the benefits in ambulation, pain, and endurance that were demonstrated in previous studies may have resulted from a placebo effect, because these studies were not placebo-controlled trials.
To further address the effects of bisphosphonates on bone, Dr Marini and her co-workers turned to a knock-in mouse model for type 4 OI, the brittle mouse. These mice were treated with subcutaneous alendronate from 2 to 14 weeks of age, killed, and compared with placebo-treated brittle mice and alendronate-treated wild-type normal mice. Alendronate had no effect on growth of either genotype. Treated brittle and wild-type mice showed an increase in BMD as measured by DXA. Bone volume per total volume more than doubled because of an increase in trabecular number, not thickness. Cortical thickness in the brittle mouse normalized. Mechanical properties were determined by assessing the 4-point bending to fracture. Stiffness significantly increased in the treated normal mice but not in the brittle mice. The “load to fracture” increased in both the wild- and brittle mice because of the increased bone volume. However, the bone became more brittle in both mice. The material strength of the bone was less than that before treatment. Finally, the osteoblast surface decreased with treatment and the morphology of the osteoblast changed, which is consistent with a direct toxic effect on cells. Dr Marini concluded that the goal of bisphosphonates in OI should be to treat long enough to reap the benefits without paying the price of the negative effects.

The final session of the workshop dealt with hormones as pharmacologic agents. Dr Mary Leonard summarized recent studies of the effects of glucocorticoid therapy in children with chronic disorders by her and her co-workers. She emphasized the complex interactions between the effects of disease-related factors, such as inflammatory cytokines, immobility, and undernutrition, and the effects of glucocorticoids. Adverse effects on bone size and strength as well as body composition may be attributed to the underlying disease for which glucocorticoid therapy is prescribed as much as to the drug itself.

Dr Madhusmita Misra examined the key role of the sex steroids as other key hormonal influences on bone. Estrogen stimulates both linear growth and bone-mass accrual, indirectly through stimulation of growth hormone and insulin-like growth factor 1 production and directly by inhibition of osteoclastic bone resorption later in puberty. Androgens also affect bone, indirectly by aromatization to estrogen and directly via the osteoblast androgen receptor to increase periosteal bone apposition, which makes the male cortices thicker. In addition, androgens directly inhibit osteoclastic bone resorption later in puberty.

Next, Dr Robert Weinstein reviewed the skeletal effects of anticonvulsant drug therapy in children. He challenged the commonly cited model that bone loss associated with these agents is caused by osteomalacia. Instead, he attributed bone loss to a high-turnover state that results from drug-induced interference with calcium absorption as well as direct effects on osteoclasts and osteoblasts. The resultant hypocalcemia can exacerbate seizures that are treated with higher doses of anticonvulsants, which sets up a vicious cycle. The important issues to be addressed in this area include that of the mechanism of anticonvulsant effects on bone cells and whether some of the newer anticonvulsants will influence ionized calcium and parathyroid hormone levels in a manner similar to older agents.

Finally, Dr Gordon Klein summarized the recent work of his group on the anabolic effects of recombinant human growth hormone (rhGH) in the treatment of bone loss after burn injury. He observed that children who received 12 months of rhGH had significantly greater increases in bone mineral content and bone area of the total body than did placebo-treated control patients. This raised the question of whether bone size or bone calcium accretion is more important in the reduction of fracture risk in this population. The other issue raised by the study was that of whether effects of growth hormone on bone are primary or secondary to the increase in lean body mass, which preceded gains in bone mineral content in the rhGH-treated group.

Collectively, these presentations reinforced the need to develop meaningful clinical end points for evaluation, to modify the available diagnostic tools to more precisely evaluate the skeleton of the growing child, and to use our knowledge of bone biology and pharmacology to more effectively intervene to prevent or reverse processes that adversely affect bone.

What follows are articles selected by the organizing committee as among the most instructive for the understanding of the interaction of chronic disease and chronic drug treatment on pediatric bone.

REFERENCES


Childhood Bone Mass Acquisition and Peak Bone Mass May Not Be Important Determinants of Bone Mass in Late Adulthood

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ABSTRACT

During childhood and adolescence, bone mass acquisition occurs primarily through skeletal growth. It is widely assumed that bone mass acquisition throughout childhood is an important determinant of the risk of osteoporosis in late adulthood; bone mass is thought to resemble a bank account in which deposits persist indefinitely. However, several well-controlled clinical studies suggest that increasing bone mass acquisition during childhood will have only transient effects. A likely explanation is that bone mass is governed by a homeostatic system that tends to return to a set point after any perturbation and, therefore, bone mass depends primarily on recent conditions, not those in the distant past. Indeed, in an animal model, we have shown evidence that bone mass acquisition in early life has no effect on bone mass in adulthood, in part because many areas of the juvenile skeleton are replaced in toto through skeletal growth. Therefore, it should not be assumed that alterations in childhood bone mass acquisition will affect bone mass many decades later in late adulthood. This issue remains open and the solution may depend on the type of childhood condition (for example calcium intake versus exercise) and its magnitude, timing, and duration. To date, both animal studies and clinical studies suggest that much of the effect of early bone mass acquisition does not persist.
Bone mass increases dramatically during childhood and adolescence, peaking in young adulthood (Fig 1A). Bone mass then plateaus and finally declines, sometimes eventuating in osteoporosis, a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. It is often assumed that bone mass acquisition throughout childhood is an important determinant of bone density and fracture risk late in life. Thus, for example, it is generally thought that optimizing calcium intake in a young child will lead to a greater peak bone mass in young adulthood and that this increment in bone mass will have a persistent effect decades later (Fig 1B). An analogy is sometimes made between bone mass and a bank account: we make deposits to our account during childhood and adolescence and withdrawals during adulthood. According to this model, greater deposits in childhood will produce a greater peak balance. Assuming that withdrawals in adulthood occur at the usual pace, the greater peak balance should delay the eventual decline into the bankruptcy of osteoporosis.

The assumption that childhood bone mass acquisition affects bone mass in the latter part of the life span is frequently repeated in review articles. For example, Heaney et al state that “achieving a high adult peak bone mass is protective against late-life fragility fractures. . . .” This same concept is asserted in the 2000 National Institutes of Health consensus statement on osteoporosis, and the assumption seems to affect public policy. The recent Surgeon General’s report on bone health states that “failure to achieve an optimized bone mass at the end of adolescence leaves an individual with much less reserve to withstand the normal losses during later life.” Educational literature produced by the Department of Health and Human Services states that “[b]etween the ages 10 to 18 is when you make the bone that must last a lifetime—this bone is known as peak bone mass. To reach the best possible peak bone mass means getting enough exercise and calcium. Bones are like a bank account—if you deposit lots of exercise and calcium now, when you are young, you will have strong bones for later in life” and, therefore, “osteoporosis is a pediatric preventable disease.”

In this article we explore the biological mechanisms responsible for bone mass acquisition during childhood that determine peak bone mass, and then we critically examine the assumption that bone mass acquisition early in life is an important determinant of bone mass in later life.

FIGURE 1
Conceptual graphs of bone mass as a function of age. A, In normal individuals, bone mass increases during childhood and adolescence, peaks in young adulthood, and then decreases in later adulthood. B, It is often assumed that an intervention (solid box) during childhood to increase bone mass acquisition will have a persistent effect on bone mass throughout life. C, However, several studies suggest that increased bone mass acquisition in childhood does not necessarily increase peak bone mass. D, Similarly, increasing peak bone mass does not necessarily result in an increased bone mass in late adulthood. Even interventions in later adulthood to increase bone mass do not necessarily have persistent effects (E); the effects may disappear with time as homeostatic mechanisms bring bone mass back toward a set point (F). Thus, bone mass may be determined primarily by recent conditions, not those in the distant past. The dashed curves represent the bone mass resulting from the intervention; the solid curves represent bone mass in the absence of intervention.
ACQUISITION OF BONE MASS IN CHILDHOOD

In adults, changes in bone mass occur primarily through remodeling, a process in which osteoclastic bone resorption is coupled with local osteoblastic bone formation. In children, bone mass is affected not only by remodeling but also by skeletal growth or modeling, a process in which bone formation and resorption are uncoupled and occur at different sites, resulting in an increase in overall bone size. The enormous increase in bone mass seen during childhood and adolescence is primarily because of this increase in bone size. Skeletal growth occurs by several different mechanisms that allow for both longitudinal and cross-sectional growth.

Longitudinal growth of long bones and vertebrae occurs through endochondral ossification. In this process, new trabecular bone is formed using a cartilaginous template generated by the growth plate. As a tubular bone elongates, older trabeculae near the center of the bone are resorbed to make room for the marrow cavity (Fig 2). More peripherally located trabeculae coalesce to form cortical bone, which causes elongation of the metaphyseal cortex. In the diaphysis, periosteal cortical bone formation coupled with endosteal cortical bone resorption lead to cross-sectional bone growth. Later in adolescence, bone formation also occurs at the endosteal surface, which further increases cortical thickness.

Skeletal growth slows with age and eventually ceases. For longitudinal bone growth, this decline seems to be attributable to a mechanism that is intrinsic to the growth plate; recent evidence suggests that growth-plate chondrocytes may have a finite proliferative capacity that is gradually exhausted. Growth-inhibiting conditions in childhood slow down proliferation and, thus, seem to conserve the proliferative capacity of the growth plate. If the growth-inhibiting condition resolves, the growth-plate chondrocytes will have retained more of their proliferative capacity than normal and, thus, will grow more rapidly than normal, resulting in catch-up growth. Whether analogous mechanisms govern periosteal bone growth is not known.

DOES EARLY BONE MASS ACQUISITION AFFECT PEAK BONE MASS?

Genetic factors account for ~50% to 85% of the variance in adult bone mineral density, depending on the site examined. Because heredity does not determine all of the variance, environmental modifications might make a substantial impact on peak bone mass. In an effort to maximize peak bone mass, public health efforts have targeted childhood as a critical time for maximizing calcium intake, weight-bearing exercises, and other bone-promoting regimens. Likewise, medical interventions that decrease bone mass are often avoided because of the concern that peak bone mass will be compromised. But, do these interventions in childhood truly affect adult bone mass?

We recently explored this question using an animal model. Young rabbits were treated between 5 and 10 weeks of age with high doses of glucocorticoid, which induced osteoporosis. The rabbits were then followed off glucocorticoid until 26 weeks of age, at which time their skeletal growth was nearing completion. Under the common assumption that bone mass is similar to a bank account, we would predict that this severe failure of bone mass acquisition during growth would have a long-lasting effect and lead to a diminished peak bone mass. However, after the glucocorticoid treatment was stopped, bone size, density, and strength recovered completely to match the values of untreated controls. Thus, early bone mass acquisition had no effect on peak bone mass. Fluorescent labeling of newly formed bone with oxytetracycline demonstrated that the recovery from osteoporosis did not occur through remodeling of osteoporotic bone. Rather, the osteoporotic bone was resorbed and replaced in toto with new healthy bone through the processes of normal longitudinal and cross-sectional skeletal growth. In the long-bone metaphysis, the osteoporotic trabecular bone formed during dexamethasone treatment was resorbed as the medullary cavity enlarged and was replaced by new bone formed by endochondral ossification at the growth plate. In the long-bone diaphysis, the periosteal bone formation rate

FIGURE 2
Schematic diagram representing the replacement of juvenile bone through skeletal growth. As the bone enlarges, new bone (black) is created by endochondral bone formation at the growth plate and periosteal bone formation at the cortex. As the marrow cavity expands, the juvenile bone (gray) is largely resorbed. Areas surrounded by dotted lines represent juvenile bone that has been resorbed.
was decreased during dexamethasone treatment but afterward rebounded above controls, normalizing cortical width. The mechanism responsible for this catch-up growth in the cortex is not known; it might be analogous to the catch-up growth that occurs at the growth plate, or it could be a result of increased mechanical load. These data in an animal model suggest that early bone mass acquisition has little effect on adult bone mass.

Human studies also suggest that alterations in bone mass acquisition during childhood may not have persistent effects. In a study by Johnston et al., identical twin pairs were randomly assigned to receive either calcium supplementation or placebo in a double-blind fashion for 3 years during childhood and adolescence. In the prepubertal children, calcium supplementation increased the gain in bone density. However, after 3 years of follow-up, the effect disappeared. Similarly, Bonjour et al. randomly assigned prepubertal girls to receive either calcium-enriched foods or placebo for 1 year. The intervention group showed a significantly greater increase in bone mass and density (average of 6 anatomic sites by dual-energy x-ray absorptiometry) at the end of treatment period and at follow-up 3 to 5 years after discontinuation. A recent report described the follow-up results at 8 years. The report focused on a posthoc division of the subjects according to menarcheal age, but in the overall subject population, the average bone density at the 6 sites was no longer significantly different between the groups.

Surprisingly, even if the calcium supplementation is not discontinued, there still may not be much effect on peak bone mass. In a recently reported study, calcium supplementation was given to girls beginning in early puberty. At the end of 4 years, a significant effect on bone mineral density was observed, but by 7 years the effect was largely lost.

Thus, clinical studies suggest that interventions to increase bone mass acquisition in childhood do not necessarily increase peak bone mass (Fig 1C). However, the persistence of effects may depend on the nature of the intervention; although calcium supplementation seems to have little long-term effect, a persistent increase in mechanical load may have a more lasting effect.

DOES PEAK BONE MASS AFFECT BONE MASS IN LATE ADULTHOOD?

Suppose that one could identify and implement an effective method to increase peak bone mass. Does it necessarily follow that this alteration in peak bone mass will have a persistent effect decades later (Fig 1B), as is commonly assumed? The veracity of this assumption seems obvious on the basis of simple mathematical reasoning. After all, bone mass at 70 years of age is the mathematical sum of peak bone mass at 30 years of age, plus all the bone that was formed between ages 30 and 70, minus all the bone that was resorbed in those years. Therefore, it would seem self-evident that increasing the peak bone mass would cause an increase in bone mass at age 70. However, this reasoning is only valid if the 3 variables in the equation (peak bone mass, subsequent bone formation, and subsequent bone resorption) are independent, which is not the case. Bone mass is governed by a homeostatic system with the set point of the system determined by genetics, mechanical load, and other environmental factors. Therefore, any perturbation in the system tends to be corrected over time. As a result, the rate of bone loss in adulthood may depend on the peak bone mass; consequently, it is not mathematically certain that increasing bone mass acquisition during childhood will necessarily lead to a greater bone mass at age 70.

If we cannot prove the importance of peak bone mass theoretically, can we prove it empirically? Several lines of evidence have been cited in support of this concept. For example, bone density tends to track along a percentile, at least in the short-term. So, if an individual has a low bone density at one point in life, he or she tends to have a low bone density later in life. If this tendency were to extend from the time of peak bone mass in early adulthood to the time of osteoporosis in late adulthood, this might suggest that peak bone mass affects later bone mass. However, association does not imply causality. The association between early bone mass and later bone mass could arise from a direct causal link between the two (Fig 3A), but it could also arise if both early bone mass and later mass are influenced by the same factors (Fig 3B). Indeed, bone mass and density throughout life are probably influenced by genetic factors and, perhaps, persistent lifestyle factors. Thus, the concordance between early bone mass and later bone mass could reflect the fact that both are influenced by a person’s genetic makeup rather than a direct causal link.
To prove a direct causal link between peak bone mass and bone mass in later life (Fig 3A), we would need 2 groups that differed only in a behavior during childhood/adolescence that affects peak bone mass; behavior in adulthood would have to be similar. We would then look at the 2 groups in late adulthood to determine whether the difference in bone mass persisted. Some observational studies have tried to address this issue. For example, in young soccer players, athletic activity is associated with increased bone density, but cessation of the athletic activity leads to increased bone loss, exceeding that of controls. This finding suggests that there is a tendency for gains not to persist but rather for the system to return toward a homeostatic set point after a perturbation (Fig 1D). In general, such studies suggest that the positive effects of physical activity on bone density may persist for years but not decades, although changes in bone size might be better preserved. However, observational studies have obvious weaknesses such as the possibility that the 2 groups might have persisting lifestyle differences that would affect the rate of bone loss through adulthood. To be sure that the 2 groups differ only in peak bone mass, individuals would need to be randomly assigned during childhood to some intervention that affects peak bone mass. Once peak bone mass is achieved, the intervention would be stopped and the subjects followed for several decades to determine if the effect persists or if it fades away as bone mass returns to a homeostatic set point. The study should be placebo-controlled and double-blind. Obviously, a prospective study of this kind, lasting decades, would be very difficult to execute. In the absence of this ideal study, we are forced to rely on indirect and imperfect evidence; therefore, any conclusions must be considered tentative.

Similar to the interventional pediatric studies that have demonstrated lack of persistent effect on bone mass, the tendency of bone to revert to a homeostatic set point after an intervention has also been seen in later adulthood. Postmenopausal women who received estrogen and then stopped the treatment did not show a persistent effect from this deposition of bone mass but rather showed a loss of the beneficial effect with time. These women demonstrated an increased rate of bone loss compared with untreated women after the intervention was stopped. In other words, the increased bone mass did not act like a deposit in a bank account (Fig 1E). Instead, the system seemed to return to its homeostatic set point (Fig 1F). Similar regression occurred after treatment with parathyroid hormone. Thus, recent conditions, not conditions in the distant past, seem to be more important determinants of bone mass. If a deposit to the “bone bank” at age 60 does not persist until age 70, can we assume that deposits in childhood will last until age 70? Thus, bone mass in later adult life may depend more on genetic factors and conditions in the latter part of adulthood, including nutritional, mechanical, pathologic, hormonal, and pharmacologic factors.

Perhaps if we began an intervention in childhood and continued it throughout life we could affect bone mass in late adulthood. However, we then must ask whether the resultant mass would be any greater than if we had started the intervention in adulthood. For example, if we are interested in optimizing bone mass at 70 years of age, would starting calcium supplementation at 3 years of age and continuing it for 67 years be any more effective than starting supplementation at 65 years of age and continuing it for 5 years? If bone mass depends primarily on recent conditions, the answer might well be no.

CONCLUSIONS
During childhood and adolescence, bone mass acquisition occurs through skeletal growth or modeling, both longitudinal growth at the growth plate and cross-sectional growth at the periosteum. It is widely assumed that bone mass acquisition throughout childhood is an important determinant of the risk of osteoporosis in late adulthood; bone mass is thought to resemble a bank account in which deposits persist indefinitely. However, this assumption cannot be proven on theoretical grounds. It is equally conceivable that increasing bone mass acquisition during childhood will have only transient effects, because bone mass is governed by a homeostatic system that tends to return to a set point after any perturbation and, therefore, bone mass depends primarily on recent conditions, not those in the distant past. Indeed, several well-controlled clinical studies have suggested that the latter alternative is true, at least in part. In an animal model, we have shown evidence that bone mass acquisition in early life has no effect on bone mass in adulthood, in part because many areas of the juvenile skeleton are replaced in toto through skeletal growth. Thus, both modeling and remodeling may erase any early effects.

It should not be assumed that alterations in childhood bone mass acquisition will affect bone mass many decades later in late adulthood. This issue remains open, and its resolution may depend on the type of childhood condition (eg, calcium intake versus exercise) and its magnitude, timing, and duration. To date, both animal and clinical studies suggest that much of the effect of early bone mass acquisition does not persist.

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Bone Accrual in Children: Adding Substance to Surfaces

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ABSTRACT

The mass of growing bones increases through changes in outer dimensions and through the net addition of tissue on inner bone surfaces. In this overview I examine bone accrual as it occurs on trabecular (inner) and periosteal (outer) surfaces. In the axial skeleton, the amount of trabecular bone increases during development, because trabeculae grow thicker as a result of bone remodeling with a positive balance. Remodeling is a process in which osteoblasts and osteoclasts are tightly linked (“coupled”) in time and space. In contrast to trabecular thickness, trabecular number and material density change little throughout development. Bone accrual on periosteal surfaces leads to an increase in bone size, which is a crucial determinant of bone strength throughout life. Periosteal osteoblasts deposit new bone on an extended surface area and over an extended period of time without being interrupted by osteoclasts. This type of bone metabolic activity is called modeling, which is much more efficient than remodeling for increasing bone mass. In the past, research has focused on bone remodeling on trabecular surfaces. However, the key to an improved understanding of bone mass and strength development in children will lie with studies on bone modeling on periosteal surfaces.
For a long time, studies on human bone development have focused on changes in bone mineral mass (expressed as bone mineral content) and bone mineral density (BMD). Nevertheless, describing bone development just in terms of changes in mass or density means looking at bones as if they were amorphous heaps of calcium and phosphorus. In reality, of course, bones are complex three-dimensional structures. Taking structural aspects into account should allow for a more realistic understanding of bone development.1,2

What are the structures on which bone tissue accrues? Schematically, new bone can be added on the outside, which makes bones bigger, or on the inside, which makes bones denser. Bones get bigger by 2 different processes: growth in length and growth in width. Bone growth in length is driven by the growth plate, whereas bone growth in width is the task of the periosteum. The inner bone surface is called the endosteal surface, which can be subdivided into the trabecular, endocortical, and intracortical surfaces.3 In this contribution, I focus on 2 sites of bone accrual: the periosteal and trabecular surfaces.

Bone Accrual on Trabecular Surfaces

At the lumbar spine, volumetric BMD, as measured by quantitative computed tomography, increases by ~25% during puberty.4 What structural changes underlie this increase? Schematically, there are 3 ways to increase trabecular BMD. First, material density might increase. Second, there might be a rise in trabecular number (ie, the trabeculae could be packed more closely together). Third, it is possible that trabeculae become thicker. Which of these 3 possibilities contributes most to the increase in lumbar spine BMD?

Material bone density cannot be measured with currently available noninvasive techniques but can be determined in histologic bone sections by using methods such as back-scattered electron-imaging analysis. With this technique, Roschger et al5 found that material trabecular bone density in the L4 vertebral body increased by only 3% from 1 to 80 years of age. Thus, material density does not seem to be a major contributor to changes in trabecular BMD during bone development.

Trabecular number, in histomorphometric terminology, reflects the number of trabeculae that a line through the bone would hit per millimeter of its length.3 Kneissel et al6 did not find any increase in trabecular number in the L4 vertebral body between 10 and 20 years of age. Thus, both trabecular number and material density cannot account for the increase in trabecular BMD during puberty. By default, then, trabecular thickening must the explanation.

I am not aware of any studies that have examined trabecular thickness in the vertebral bodies of children and adolescents. However, our own studies7 of a different part of the axial skeleton, the ilium, indeed revealed an increase in trabecular thickness during bone development. Similar to vertebral bodies, no change in trabecular number was found throughout the growing period. Dynamic histomorphometric measurements suggested that the increase in trabecular thickness was attributable to remodeling with a positive balance,7 which means that during each remodeling cycle osteoblasts add more bone than was previously resorbed by osteoclasts. The difference is small, however, and only leads to a gain of a few micrometers of trabecular thickness per remodeling cycle. As in growing children each location on the iliac trabecular surface undergoes a remodeling cycle every 9 to 10 months, on average, remodeling with a positive balance results in a very slow and gradual increase in trabecular BMD.

Bone Accrual on Periosteal Surfaces

Bone growth in width is much less well characterized than growth in length, although it is of paramount importance for bone stability. If bones just grew in length without increasing in size, they would become unstable and break at some point.5 The bending strength of an elongated structure such as a long-bone diaphysis is related to its diameter raised to the third power (Fig 1). In contrast, bending strength is inversely related to length raised to the third power.6 Thus, bone growth in length and growth in cross-sectional size have opposite effects on a bone’s ability to withstand mechanical loads. Growth in size, therefore, must be closely linked to growth in length. How this works is unknown. After the growth period, bone size changes only slowly. Consequently, bone growth in size is one of the most important determinants of bone strength throughout life.9

A bone’s cross-sectional size increases through the action of osteoblasts that add mineralized tissue on the outer (periosteal) bone surface, a process called periosteal apposition.10 The periosteum surrounds the bone tube. The action of osteoblasts that add mineralized tissue on the outer (periosteal) bone surface, a process called periosteal apposition.10 The periosteum surrounds the bone tube.
like a stocking, which in children is thick and is only loosely attached to the diaphysis. Toward the bone ends, the periosteum continues directly into the perichondral ring that encircles the periphery of the growth plate. The periosteum and perichondrium are both firmly anchored to the epiphysis.11

On the microscopic level, the periosteum consists of 2 readily distinguishable layers. The outer layer is composed mainly of fibrous tissue, and the inner layer, called the cambium layer, harbors osteogenic cells. These osteogenic cells have not been characterized in any great detail, and little is known about their differentiation pathways.12 In 2-week-old rabbits, osteoblasts remain active on the periosteal surface for only 3 days.13 They then seem to lose steam, get buried in newly deposited bone matrix, and turn into osteocytes.

Histomorphometric studies of rib and iliac bone have yielded the expected result that periosteal bone formation is much more active in children than in adults.7,14,15 However, there may be a more fundamental difference between periosteal bone metabolism in children and adults. In children, bone formation is continuous, which is the hallmark of modeling.7,16 In adults, periosteal bone may undergo cyclical resorption and formation, which is characteristic of remodeling.17,18 Because remodeling is the process responsible for bone loss in adults, it is widely studied in the field of osteoporosis research. Bone modeling, however, has received little attention until now.

Most of the available information on human periosteal bone growth is based on radiographic studies, and most were performed at the midshaft of long bones. Studies by Garn et al19,20, which were performed using this approach, are widely cited classics. They measured the width of the second metacarpal in a large number of healthy subjects. The corresponding periosteal apposition rates showed changes with age that resemble percentile charts for height velocity (Fig 2). Growth is rapid during early life but then continuously slows down until it reaches a nadir during early school age. This is followed by a pubertal peak, after which periosteal growth (almost) comes to a standstill.

It is clear that wider bones must have higher midshaft periosteal apposition rates, because this is how they become wider. For example, during male puberty the estimated peak periosteal apposition rate of the metacarpal is ~0.5 μm/day, but it is close to 2 μm/day at the midshaft humerus.21 What is less widely appreciated is that periosteal growth is not necessarily synchronized between bones. For example, in 3-month-old infants, the humerus grows in width one-third faster than the femur (Fig 3). At the age of 1 year, the 2 bones expand at approximately the same rate, whereas at 33 months of age, periosteal apposition is almost 4 times as fast at the femur as it is at the humerus. At 5 years of age, this difference in periosteal apposition rate between the 2 bones shrinks to 25% in favor of the femur. These differences in bone growth in width between the humerus and femur mirror the mechanical usage of these extremities during development between 1 and 4 years of age.22 When infants start to walk, the femur is exposed to much higher forces and gets stronger quickly. At the same time, the humerus is used less and less for locomotive purposes and, accordingly, humerus strength increase is slow.

**THE CONTROL OF PERIOSTEAL BONE GROWTH**

Research on the regulation of periosteal bone development has focused mainly on systemic hormones. A number of elegant studies have demonstrated that estrogen inhibits, and androgen and growth hormone stimulate, periosteal apposition at diaphyseal bone sites.23–25 However, this focus on systemic factors should not make us lose sight of the fact that periosteal bone...
development is site specific, whereas systemic hormones and nutrition are blind to structure. Systemic factors, therefore, cannot be the main determinants of what is going on at the periosteum. Clearly, local regulation must predominate, albeit modulated by systemic agents.

One of these site-specific factors is the mechanical load that acts on a bone. For example, when the radius of young pigs is overloaded by partially removing the ulna, the radius is strengthened by rapid periosteal apposition. When plastic surgeons transplant a fibula to replace a tibia that has been destroyed by tumor or infection, the fibula quickly hypertrophies and comes to replace a tibia that has been destroyed by tumor or infection. Conversely, disorders that result in removal of mechanical stimulus during growth, such as cerebral palsy, spina bifida, or poliomyelitis, lead to thin bones in the affected segments.

CONCLUSIONS
In the past, research on bone metabolism and development has focused on events that occur on trabecular surfaces. However, in children and adolescents, the changes on these surfaces are relatively small compared with the marked increase in cross-sectional bone size. Much more bone accrues through periosteal bone modeling than through trabecular remodeling. The key to an improved understanding of bone mass accrual in children, therefore, lies with studies on bone modeling on periosteal surfaces.

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Calcium and Vitamin D: What Is Known About the Effects on Growing Bone

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ABSTRACT

The objective of these investigations was to determine if the receptor-dependent effects of 1,25-dihydroxyvitamin D were essential for normal skeletal growth. Mice with targeted ablation of the vitamin D receptor were engineered, and the skeletal consequences of vitamin D receptor ablation were studied in the presence of normal and abnormal mineral ion homeostasis. Prevention of abnormal mineral ion homeostasis resulted in the development of a normal skeleton in the absence of a functional vitamin D receptor. The metabolic cause of rickets was found to be hypophosphatemia. The major receptor-dependent actions of 1,25-dihydroxyvitamin D on skeletal development are indirect and are a reflection of the role of this hormone on intestinal calcium absorption.

Key Words
vitamin D receptor, ablation, hypophosphatemia, rickets

Abbreviations
VDR—vitamin D receptor
MGP—matrix gla protein

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275); published in the public domain by the American Academy of Pediatrics.
**The active metabolite** of vitamin D, 1,25-dihydroxyvitamin D, is thought to exert its effects by binding to the vitamin D receptor (VDR), a member of the nuclear receptor family of ligand-dependent transcription factors. The receptor-dependent actions of 1,25-dihydroxyvitamin D have been shown to promote intestinal calcium absorption, suppress PTH gene transcription, regulate the expression of bone-matrix proteins, and promote osteoclast differentiation by inducing the expression of RANK (receptor activator of nuclear factor κB) ligand. Although these studies have demonstrated that 1,25-dihydroxyvitamin D has several actions that contribute to the regulation of skeletal and mineral ion homeostasis, questions remained as to whether the actions of 1,25-dihydroxyvitamin D were essential and whether the in vivo consequences of vitamin D deficiency were a direct result of impaired hormone-dependent receptor actions.

To address these issues, studies were performed in VDR-null mice. These mice, which have no detectable receptor protein, are a phenocopy of the human disorder hereditary vitamin D–resistant rickets. They are phenotypically normal at birth but develop secondary hyperparathyroidism the third week of life as a result of impaired intestinal calcium absorption. The increased PTH levels lead to hypophosphatemia by 21 days of age because of PTH-dependent urinary phosphorus losses. The skeletal manifestations observed in both mice and humans with VDR mutations are similar to those seen in people with vitamin D deficiency. Rickets, characterized by a dramatic expansion of the growth plate and hypomineralized flared metaphyses, is observed by the fourth week of life. Osteomalacia is also seen, which leads to impaired biomechanical properties of the skeleton.

To dissect which of these skeletal manifestations of VDR ablation were a direct consequence of impaired hormone-dependent receptor actions versus the resultant abnormalities in mineral ion homeostasis, VDR-null mice were placed on a diet enriched in calcium, phosphorus, and lactose before the development of secondary hyperparathyroidism. This diet prevented the development of abnormal mineral ion homeostasis, thus allowing us to determine if the receptor-dependent actions of 1,25-dihydroxyvitamin D were essential for skeletal maturation. Unlike VDR-null mice with abnormal mineral ion levels, the mice on this special diet did not develop rickets or osteomalacia. It was notable that both histomorphometric and biomechanical analyses failed to identify a specific skeletal abnormality that was a direct consequence of VDR ablation. Thus, the major contribution of 1,25-dihydroxyvitamin D to skeletal maturation is to promote intestinal calcium absorption and provide an optimal metabolic environment for skeletal growth.

Although 1,25-dihydroxyvitamin D has been shown to modulate gene expression and differentiation of growth-plate chondrocytes in vitro, our investigations demonstrated that VDR-null mice, when treated with a diet that normalizes mineral ion levels, do not have a detectable skeletal defect. Therefore, we undertook investigations to clarify the basis for the growth-plate abnormality in the VDR-null mice. Within 2 days of the development of hyperparathyroidism, we observed an expansion in the hypertrophic chondrocyte layer of the VDR-null mice. On the basis of the work of other investigators, which demonstrated that extracellular calcium promotes expression of markers of terminal chondrocyte differentiation, we addressed the hypothesis that abnormal mineral ion homeostasis led to impaired chondrocyte differentiation. However, markers of chondrocyte differentiation were unaltered, as were chondrocyte proliferation and expression of vascular endothelial growth factor, a key signal for vascular invasion, a process that is required for replacement of the terminally differentiated hypertrophic chondrocytes by bone. A marked increase in expression of matrix gla protein (MGP), a potent inhibitor of matrix mineralization, was observed. We addressed whether MGP played a physiological role in expansion of the late hypertrophic chondrocyte layer by making the VDR-knockout mice null for MGP as well. This did not normalize the growth-plate phenotype. Because the cellular basis for the rachitic changes involved expansion of the terminally differentiated, osteopontin-expressing late hypertrophic chondrocytes, we evaluated apoptosis, the final stage in differentiation of these cells. Histologic analyses demonstrated a marked decrease in apoptosis of the late hypertrophic chondrocytes in the rachitic VDR-null mice, thus clarifying the cellular basis for this abnormality.

Although these studies were critical in identifying the cellular basis for the rachitic changes, they did not address the underlying pathophysiology that led to this abnormality. Therefore, we performed studies to demonstrate that normalizing mineral ion homeostasis in the VDR-null mice, which is associated with normal growth-plate histology, normalizes apoptosis. These results raised the question of whether the impaired apoptosis was secondary to hypocalemnia, hyperparathyroidism, or hypophosphatemia. To address this issue, studies were performed to characterize the growth-plate phenotype in 2 additional murine models: diet-induced hypophosphatemia/hypercalcemia and the **hyp** mouse (which has a mutation in the **PHEX** gene and is the murine model for the human disease X-linked hypophosphatemia). The mice with hypophosphatemia in the presence of hypercalcemia (and suppressed PTH levels) and the **hyp** mice (normal calcium and PTH levels) both demonstrated expansion of the late hypertrophic chondrocyte layer associated with impaired apoptosis of these cells, which points to hypophosphatemia as the common etiologic factor.
Interpretation of studies in the hyp mice are not straightforward because of the possibility that mutation of the PHEX gene may lead to an intrinsic chondrocyte defect, analogous to the osteoblast defect that has been reported.\textsuperscript{11,12} Therefore, to address whether hypophosphatemia, as opposed to an intrinsic chondrocyte defect, was the primary cause of the rachitic changes in the hyp mice, the growth plate of hyp mice with normal mineral ion homeostasis was examined. Because the serum phosphate of the hyp fetuses is indistinguishable from that of their wild-type littermates, examining the growth plate before and after birth permitted investigations directed at correlating the development of rickets with that of hypophosphatemia. It is interesting to note that at 18.5 days of embryonic life, correlating with a normal phosphorus level, the growth-plate phenotype of the hyp mice was normal both histologically and by terminal deoxynucleotidyltransferase-mediated 2′,3′-dideoxuridine 5′-diphosphate nick end labeling (TUNEL) evaluation of apoptotic cells. The development of rachitic changes in this model paralleled the development of impaired chondrocyte apoptosis and hypophosphatemia, which lends further credence to the hypothesis that hypophosphatemia leads to rickets by impairing apoptosis of late hypertrophic chondrocytes.\textsuperscript{10} It is interesting to note that in the models examined, impaired apoptosis was observed at a time when there was still considerable mineralized matrix surrounding the late hypertrophic chondrocytes, which suggests that circulating, rather than locally deposited, phosphate is the critical determinant of apoptosis. Supporting this hypothesis is the observation that tissue-nonspecific alkaline phosphatase–knockout mice (a model for the human disease hypophosphatasia) have normal circulating phosphate levels, have markedly impaired matrix mineralization, and do not develop rickets.\textsuperscript{13}

Because hypophosphatemia impairs hypertrophic chondrocyte apoptosis in vivo, which leads to rickets, the pathway by which phosphate mediates chondrocyte apoptosis was examined. Studies by other investigators in an avian chondrocyte culture system demonstrated that phosphate induces chondrocyte apoptosis in a dose-dependent manner.\textsuperscript{14–16} A primary murine chondrocyte culture model was used to determine if phosphate-mediated chondrocyte apoptosis involved the extrinsic (membrane) or intrinsic (mitochondrial) apoptotic pathway.\textsuperscript{17} These studies demonstrated that phosphate treatment of hypertrophic chondrocytes led to activation of caspase-9, a mediator of the mitochondrial apoptotic pathway. Furthermore, inhibition of mitochondrial permeability transition (an initial step in activation of the mitochondrial apoptotic pathway that involves the inner-membrane pathway) with cyclosporin A inhibited caspase-9 activation.\textsuperscript{18} It is interesting to note that activation of caspase-9 by phosphate was cell type– and differentiation stage–specific in that phosphate did not activate caspase-9 in 3T3 fibroblasts or in primary chondrocytes that had not undergone hypertrophic differentiation. To demonstrate that activation of the mitochondrial apoptotic pathway was critical for hypertrophic chondrocyte apoptosis in vivo, wild-type mice were treated with caspase inhibitors for 6 days. These studies demonstrated that inhibition of caspase-9 in vivo leads to expansion of the late hypertrophic chondrocytes of wild-type mice, demonstrating a role for the mitochondrial apoptotic pathway in growth-plate maturation in vivo.

Although calcium has been shown to promote chondrocyte differentiation, terminal differentiation of hypertrophic chondrocytes leading to apoptosis depends on adequate levels of circulating phosphate. Thus, the receptor-dependent actions of 1,25-dihydroxyvitamin D are critical for optimal intestinal calcium absorption, which provides an optimal metabolic environment for skeletal mineralization.

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Assessment of Bone Acquisition in Childhood and Adolescence

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ABSTRACT

Availability, ease of use, relative low cost, and minimal radiation exposure have made dual-energy x-ray absorptiometry the most widely used technique worldwide to obtain bone measurements for both research and clinical purposes in pediatric populations. However, errors related to growth and maturity significantly diminish the accuracy of dual-energy x-ray absorptiometry bone measurements. Several investigators have found that dual-energy x-ray absorptiometry in children frequently leads to a misdiagnosis of osteoporosis and an underestimation of the amount of bone. In this regard, a recent official position paper by the International Society for Clinical Densitometry states that subjects <20 years of age should not be given a diagnosis of osteoporosis on the basis of dual-energy x-ray absorptiometry criteria. Nevertheless, the increased awareness that osteoporosis has its antecedents in childhood and the demand for examinations of bone acquisition and response to therapy stress the urgent need to improve the value of dual-energy x-ray absorptiometry measurements for children.

Key Words
dual-energy x-ray absorptiometry, DXA, osteoporosis, pediatrics, QCT

Abbreviations
BMC—bone mineral content
DXA—dual-energy x-ray absorptiometry
BMD—bone mineral density
aBMD—areal bone mineral density
CT—computed tomography
QCT—quantitative computed tomography

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275) published in the public domain by the American Academy of Pediatrics
There are 2 main reasons for measuring bone mineral content (BMC) in children: to quantify the deficits in bone mineral associated with the various disorders that cause osteopenia in children and to improve our understanding of the childhood antecedents of osteoporosis, a condition that happens to manifest itself in elderly subjects. Available data suggest that the genetic susceptibility to osteoporosis may be detectable in early childhood. This notion is supported by studies that have shown that there is a strong resemblance between mother-daughter bone traits and that this resemblance is present even before the daughters have begun puberty. Additional support comes from the evidence that some genes associated with the normal variations in bone mass in elderly women may also be related to variations in bone density in children. If bone loss were the exclusive determinant of late-life bone mass, one would not expect such a strong resemblance in bone traits between girls and their mothers or in the association between candidate genes and bone mass in childhood. Peak bone mass, a major determinant of the risk for osteoporosis and fractures in the elderly population, is largely achieved at the end of sexual development in the lumbar spine and the femur. By the age of 16 years, most children have completed sexual maturity, and studies have shown that bone-mass values in girls by this age are equal to or greater than that of their premenopausal mothers.

Currently, the most commonly used quantitative radiologic method for assessing bone mass in elderly patients is dual-energy x-ray absorptiometry (DXA). This technique is also increasingly used in children because of the growing awareness that osteoporosis has its antecedents in childhood. This has increased the demand for examinations of bone acquisition and response to therapy in pediatric populations. Early bone health is key to the achievement of high peak bone mass in young adulthood and serves as the “bone bank” for the remainder of adult life. Support for this concept comes from data that indicate a strong resemblance in bone mass between mothers and prepubertal daughters and that candidate genes associated with osteoporosis and fractures in elderly patients are also associated with low bone mass in childhood. Peak bone mass may be compromised by chronic malnutrition, inactivity, teenage pregnancy, hypogonadism, chronic steroid exposure, and a multitude of pediatric diseases.

The need for accurate measurements of bone density in children is underscored by an online search for “bone mineral density children” on the National Library of Medicine’s PubMed Web site, which resulted in 1784 hits from 1990 to 2004. During this same period of time, a search for “bone density children” revealed 496 awarded grants on the National Institutes of Health CRISP (Computer Retrieval of Information on Scientific Projects) Web site (available at http://crisp.cit.nih.gov). In the year 2004 alone, 43 grants were funded by the National Institutes of Health to study bone accrual in pediatric cohorts with DXA values as major outcome measures; 15 clinical pediatric trials using DXA to monitor response are currently listed at www.ClinicalTrials.gov. Until 1997, clinical trials generally included only adults. However, the Food and Drug Administration is now encouraging therapeutic interventions to be tested in children, and many more trials involving children are under way. Accurate outcome measures are needed for pediatric interventions that are aimed at enhancing bone acquisition during growth.

At present, however, the interpretation of DXA bone studies is considerably more challenging in children than it has been in adults. Several investigators have found that osteoporosis in children is frequently misdiagnosed with DXA measures. Indeed, the American College of Radiology and the International Society for Clinical Densitometry have advised against the use of World Health Organization criteria for the classification of osteopenia and osteoporosis in adults to diagnose osteoporosis in children. Specifically, the official position of the International Society for Clinical Densitometry on the diagnosis of osteoporosis in children (males and females <20 years old) is: “The [World Health Organization] classification should not be applied to children. T-scores should not be used in the interpretation of DXA measures in children; Z-scores should be used instead. The diagnosis of osteoporosis in children should not be made on the basis of densitometric criteria alone. Terminology such as low bone density for chronological age may be used if the Z-score is below −2.0. Z-scores must be interpreted in the light of the best available pediatric databases of age- and gender-matched controls. The reference database should be cited in the report. Spine and total body are the preferred skeletal sites for measurement. The value of BMD [bone mineral density] to predict fractures in children is not clearly determined. There is no agreement on standards for adjusting BMD or BMC for factors such as bone size, pubertal stage, skeletal maturity, and body composition. If adjustments are made, they should be clearly stated in the report.”

The 3 main limitations of DXA measurements in children are (1) the current lack of a standardized pediatric normative database, (2) the lack of a meaningful clinical outcome measure related to DXA values in children, and (3) inaccuracies resulting from growth-related variations in bone and body size and composition. The first 2 limitations are being addressed by the Bone Mineral Density in Childhood Study, which is sponsored by the National Institute of Child Health and Human Development. This study involves 5 clinical centers that have recruited 1530 boys and girls aged 6 to 16 years for longitudinal bone studies. Subjects undergo a baseline and 3 consecutive annual evaluations that include DXA measurements, a bone-age radiograph of the hand, a
physical examination to determine the stage of sexual maturation, and stadiometer-measured height and weight. The longitudinal measurements will determine the degree of tracking of BMC and areal BMD (aBMD) throughout growth and help to establish the constancy of a child’s expected measures relative to population percentiles. Previous studies using quantitative computed tomography (QCT) have shown strong correlations between prepubertal and postpubertal bone-mass measurements, which suggests that bone traits can be tracked throughout adolescence. Establishing whether DXA values also retain their rank order across time will help in the identification of those children who are prone to develop low values for peak bone mass and may be at greater risk for osteoporosis later in life.

The third major limitation of pediatric DXA bone determinations, inaccuracies resulting from growth-related variations in bone and body size and composition, has not yet been addressed. Bone-mass measurements using DXA are based on a two-dimensional projection of a three-dimensional structure. The results are influenced by many skeletal and extraskeletal parameters including the size of the bone, the volume (tissue density) of the bone, and the material density of the bone being examined, as well as the amount and distribution of soft tissues around the bone. The inability of DXA to account for the influence of variations in these anatomic measures markedly hinders the accuracy and reproducibility of bone determinations in the growing skeleton. Multiple correction factors have been suggested in an attempt to overcome the influence of vertebral size on DXA measurements of the axial skeleton. Carter et al16 introduced a general approach for estimating a volumetric density that reduces the influence of bone size. Their approach was based on the concept of geometric similarity, which assumes that the skeleton scales proportionately in all directions. This implies that all lengths are proportional to each other and that areas are proportional to lengths squared. Bone thickness, therefore, should be proportional to other lengths or to the square root of a bone’s projected area, bone width, and subject height.16,20 Similar approaches used by other investigators have assumed that the vertebrae have specific shapes such as a cube,16,21 a cylinder with a circular base,22 or a cylinder with an elliptic base.23,24 Although all of these approaches are likely to reduce the effects of bone size on DXA measurements, it is not known which ones provide the best correction for a particular pediatric population. Moreover, because the shape of the vertebrae changes with age, corrections should be growth and maturity specific.

DXA values are also influenced by the unknown composition of soft tissues in the beam path of the region of interest.25,26 Corrections for the soft tissues are based on the assumption that the proportion of lean tissue and fat is the same for the beam paths beside the bone and those through the bone. If the fat and lean-tissue proportion is the same for beam paths that contain no bone as for beam paths that traverse the bone, the calculated amount of bone is accurate.27 Unfortunately, marked changes in DXA measurements are observed if fat is distributed inhomogeneously around the bone measured.28 It has been estimated that inhomogeneous fat distribution in soft tissues resulting in a difference of a 2-cm fat layer between the soft-tissue and bone areas will influence DXA measurements by 10%.29 Soft-tissue–related errors especially limit comparative studies on the effects of obesity, malnutrition, anorexia nervosa, lactation, puberty, etc, on aBMD values. In addition, longitudinal studies using DXA are subject to considerable error, because aBMD measurements may reflect changes in body size and composition more than true changes in bone density.27 It should be noted that although the precision of spinal DXA aBMD measurements has been reported to be 0.7% to 1.7%, the long-term reproducibility of these measures in children is difficult to determine, because we do not know which anatomic variable most influences the measure.28

To assess the influence of growth and development on DXA bone-mass measurements, comparative studies were performed by using QCT, which measures volumetric BMD and is not influenced by bone size.29 Among healthy children, measurements of spine BMC using DXA and QCT were highly correlated (Fig 1), but there was a far weaker relationship between aBMD (g/cm²) and volumetric BMD (g/cm³) using QCT. In fact, DXA aBMD had a stronger correlation with QCT measurements of vertebral volume (r² = 0.68) than with density. When subjects in Tanner stages 1 to 3 were considered separately from subjects in Tanner stages 4 to 5, correlations for the density were particularly poor for the less mature subjects even after correction with geometric formulas. It should be stressed that, in prepubertal children and those in the early stages of sexual development, there was no association between DXA aBMD and QCT volumetric BMD measures.

The relation between DXA and QCT z scores (defined as the number of SDs the aBMD or volumetric bone density is above or below the mean for age-matched controls) was also compared in 400 children and adolescents (100 each of healthy and sick boys and girls).30 A significant linear relationship was observed between 2 scores (defined as the number of SDs the aBMD or volumetric bone density is above or below the mean for age-matched controls) was also compared in 400 children and adolescents (100 each of healthy and sick boys and girls).30 A significant linear relationship was observed between zDXA and zQCT (r² = 0.39; P < .0001) (Fig 2). Results for the subgroups divided by health status (healthy or sick) and gender (boys or girls) were similar to the overall results (r² values of 0.27–0.48). When DXA z scores were used to predict QCT z scores below −2.0, sensitivity and specificity were reasonable, and the negative predictive value was extremely high. However, the positive predictive value was low. This was true regardless of
whether all subjects were analyzed together or sick and healthy subjects were analyzed separately.

For the subjects who were classified differently by QCT and DXA, many more were identified as having low bone density by DXA (58 of 400) than by computed tomography (CT) (7 of 400). Of the 58 subjects who were identified by DXA only, most were small for their age (<5th percentile) in terms of height (30 of 58 [52%]), weight (22 of 58 [38%]), or both height and weight (17 of 58 [29%]). The results of the current study, however, indicate that DXA measures of aBMD underestimate bone accretion as assessed by QCT in children and adolescents.

On average, 3 times as many subjects were determined to have low bone density (z score less than −2.0 for chronological age) by DXA than by QCT; this was true for both healthy and sick children. We found that although DXA and QCT z scores are related, almost 50% of the variability remains even after age and anthropometric measures are taken into account.

A critical question is whether DXA or QCT is a better surrogate measure of bone fragility in childhood. This issue remains unresolved because there are too few data linking either densitometry measure to fracture risk in children. Bone strength is determined not only by bone mass (such as BMD) but also by the size, geometry, turnover, and microarchitecture of bone. For this reason, it is possible that the influence of bone size (captured better by BMD) is more highly correlated with bone fragility. The choice of an appropriate surrogate measure for fracture is a key area of controversy that must be resolved when planning bone-health–intervention studies in pediatric populations.

In conclusion, skeletal mass is accrued throughout childhood and adolescence and is largely determined by genetic and/or familial factors. Gonadal steroids, physical activity, and dietary intake are key to bone acquisition throughout growth. Bone mass measurements using DXA, although currently limited in some respects for pediatric applications, should be optimized for the assessment of health strategies to improve a child’s skeletal status and growth.

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Bisphosphonates: Mode of Action and Pharmacology

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ABSTRACT

The profound effects of the bisphosphonates on calcium metabolism were discovered over 30 years ago, and they are now well established as the major drugs used for the treatment of bone diseases associated with excessive resorption. Their principal uses are for Paget disease of bone, myeloma, bone metastases, and osteoporosis in adults, but there has been increasing and successful application in pediatric bone diseases, notably osteogenesis imperfecta. Bisphosphonates are structural analogues of inorganic pyrophosphate but are resistant to enzymatic and chemical breakdown. Bisphosphonates inhibit bone resorption by selective adsorption to mineral surfaces and subsequent internalization by bone-resorbing osteoclasts where they interfere with various biochemical processes. The simpler, non–nitrogen-containing bisphosphonates (eg, clodronate and etidronate) can be metabolically incorporated into nonhydrolysable analogues of adenosine triphosphate (ATP) that may inhibit ATP-dependent intracellular enzymes. In contrast, the more potent, nitrogen-containing bisphosphonates (eg, pamidronate, alendronate, risedronate, and zoledronate) inhibit a key enzyme, farnesyl pyrophosphate synthase, in the mevalonate pathway, thereby preventing the biosynthesis of isoprenoid compounds that are essential for the posttranslational modification of small guanosine triphosphate (GTP)-binding proteins (which are also GTPases) such as Rab, Rho, and Rac. The inhibition of protein prenylation and the disruption of the function of these key regulatory proteins explains the loss of osteoclast activity. The recently elucidated crystal structure of farnesyl diphosphate reveals how bisphosphonates bind to and inhibit at the active site via their critical nitrogen atoms. Although bisphosphonates are now established as an important class of drugs for the treatment of many bone diseases, there is new knowledge about how they work and the subtle but potentially important differences that exist between individual bisphosphonates. Understanding these may help to explain differences in potency, onset and duration of action, and clinical effectiveness.
The discovery and development of the bisphosphonates as a major class of drugs for the treatment of bone diseases was recently reviewed and represents a fascinating story that has its origins in studies of biological calcification processes. There are many books and review articles available that describe the chemistry, pharmacology, and clinical applications of bisphosphonates.

It had been known since the 1930s that trace amounts of polyphosphates were capable of acting as water softeners by inhibiting the crystallization of calcium salts, such as calcium carbonate, and in the 1960s Fleisch et al. showed that inorganic pyrophosphate, a naturally occurring polyphosphate and a known byproduct of many biosynthetic reactions in the body, was present in serum and urine and could prevent calcification by binding to newly forming crystals of hydroxyapatite. It was proposed that inorganic pyrophosphate (PPI) might be the body’s own natural “water softener” that normally prevents calcification of soft tissues and regulates bone mineralization. It subsequently became clear that calcification disorders might be linked to disturbances in PPI metabolism. The first example was an inherited disorder, hypophosphatasia, in which lack of alkaline phosphatase is associated with mineralization defects of the skeleton and elevated PPI levels, indicating that alkaline phosphatase is probably the key extracellular enzyme responsible for hydrolyzing pyrophosphate.

Attempts to exploit these concepts by using pyrophosphate and polyphosphates to inhibit ectopic calcification in blood vessels, skin, and kidneys in laboratory animals were successful only when the compounds were injected. Orally administered pyrophosphate and polyphosphates were inactive because of their hydrolysis in the gastrointestinal tract. During the search for more stable analogues of pyrophosphate that might also have the antimineralization properties of pyrophosphate but would be resistant to hydrolysis, several different chemical classes were studied. The bisphosphonates (at that time called diposphonates), characterized by P-C-P motifs, were among these classes.

Like pyrophosphate, bisphosphonates had high affinity for bone mineral and were found to prevent calcification both in vitro and in vivo but, unlike pyrophosphate, were also able to prevent experimentally induced pathologic calcification when given orally to rats in vivo. This property of being active by mouth was key to their future use in humans.

In these early studies bisphosphonates were shown not only to prevent the experimentally induced calcification of many soft tissues, including skin, kidneys, and blood vessels in vivo but, with some of the compounds (eg, etidronate), to also inhibit mineralization of ectopic bone as well of normal calcified tissues such as bone and cartilage. Bisphosphonates seem to prevent calcification by physicochemical mechanisms that produce direct impairment of the calcification process by acting as crystal poisons after adsorption to mineral surfaces rather than by effects on the deposition of matrix.

Perhaps the most important step toward the future use of bisphosphonates occurred when we found that bisphosphonates, as we had already shown for PPI, also had the novel property of being able to inhibit the dissolution of hydroxyapatite crystals. This finding led to studies to determine if they might also inhibit bone resorption, which they did in many different experimental models. In growing intact rats, the bisphosphonates block the removal of both bone and cartilage, thus retarding the modeling of the metaphysis, which becomes club-shaped and radiologically denser than normal. This effect is the basis of the Schenk model and is a phenomenon of interest in pediatrics because it is also observed in children who are treated with high doses of bisphosphonates.

The bisphosphonates are also effective in preventing bone destruction in a number of animal models of human disease, such as immobilization osteoporosis, and the prevention of bone loss associated with ovariectomy. If not given in excess, bisphosphonates do not impair bone growth and can maintain or improve the biomechanical properties of bone in both normal animals and experimental models of osteoporosis.

In general, there is a good correlation between potency and structure-activity relationships in vitro and in vivo. In the presence of bisphosphonates, isolated osteoclasts form fewer and smaller erosion cavities on various mineralized matrices in vitro.

**Pharmacology and Cellular Actions**

Etidronate was the first bisphosphonate to be used in humans for fibro dysplasia ossificans progressiva and Paget disease. Once the potential clinical value of bisphosphonates had been appreciated, research efforts were devoted to the development of compounds with a more powerful antiresorptive activity but without a corresponding ability to inhibit mineralization. With compounds such as etidronate there was only a 10- to 100-fold difference between doses that inhibit mineralization compared with doses that reduce bone resorption. Enhancing this window was readily achieved, and many hundreds of bisphosphonates have been synthesized; more than a dozen have been used in humans. With the development of bisphosphonates that were more potent inhibitors of bone resorption, these dose differences widened to several orders of magnitude, which meant that inhibition of skeletal mineralization observed with etidronate ceased to be a major clinical concern. The gradation of potency evaluated in the animal models corresponded quite well with that found in humans, although the differences in potency are much smaller in humans. Bisphosphonates accumulate in bone, so it is impor-
tant to know what happens during long-term administration. From a clinical point of view, it is reassuring that the inhibition of bone resorption reaches a new steady-state level rather than becoming progressively lower, even when the compounds are given continuously. The level of suppression depends on the administered dose and has also been observed in humans. There seems to be no progression of the antiresorptive effect with time, which suggests that the bisphosphonate buried in the bone is inactive for at least as long as it remains buried there. This also means that within the therapeutic dosage range, there is little risk of a continuous and progressive decrease in bone turnover in the long run that might lead to an increase in bone fragility. An additional important pharmacologic property of bisphosphonates is that the total dose administered is a major determinant of their effects. This has been well studied for ibandronate and zoledronate. In both cases the same inhibition of bone resorption has been documented regardless of whether the bisphosphonate was given in small frequent (eg, daily) doses compared with larger doses given less frequently. This was the basis for the development of intermittent-dosing regimens in humans.

The pronounced selectivity of bisphosphonates for bone rather than other tissues is the basis for their value in clinical practice. Their preferential uptake by and adsorption to mineral surfaces in bone bring them into close contact with osteoclasts. During bone resorption, bisphosphonates are probably internalized by endocytosis along with other products of resorption. Many studies have shown that bisphosphonates can affect osteoclast-mediated bone resorption in a variety of ways, including effects on osteoclast recruitment, differentiation, and resorptive activity, and may induce apoptosis.

Because mature, multinucleated osteoclasts are formed by the fusion of mononuclear precursors of hematopoietic origin, bisphosphonates could also inhibit bone resorption by preventing osteoclast formation, in addition to affecting mature osteoclasts. In vitro, bisphosphonates can inhibit dose-dependently the formation of osteoclast-like cells in long-term cultures of human bone marrow. In organ culture, also, some bisphosphonates can inhibit the generation of mature osteoclasts, possibly by preventing the fusion of osteoclast precursors.

It is likely that bisphosphonates are selectively internalized by osteoclasts rather than other cell types because of their accumulation in bone and the endocytic activity of osteoclasts. During the process of bone resorption, the subcellular space beneath the osteoclast is acidified by the action of vacuolar-type proton pumps in the ruffled border of the osteoclast membrane. The acidic pH of this microenvironment causes dissolution of the hydroxyapatite bone mineral, whereas the breakdown of the extracellular bone matrix is brought about by the action of proteolytic enzymes, including cathepsin K. Because bisphosphonates adsorb to bone mineral, especially at sites of bone resorption where the mineral is most exposed, osteoclasts are the cell type in bone most likely to be exposed to the highest concentrations of free, non–mineral-bound bisphosphonate as a result of the release of the bisphosphonate from bone mineral in the low-pH environment beneath osteoclasts. It has been estimated that pharmacologic doses of alendronate that inhibit bone resorption in vivo could give rise to local concentrations as high as 1 mM alendronate in the resorption space beneath an osteoclast, which is much higher than the concentrations of bisphosphonates required to affect osteoclast morphology and cause osteoclast apoptosis in vitro.

In contrast to their ability to induce apoptosis in osteoclasts, which contributes to the inhibition of resorptive activity, some experimental studies suggest that bisphosphonates may protect osteocytes and osteoblasts from apoptosis induced by glucocorticoids. Recent evidence suggests that the inhibition of osteocyte apoptosis by bisphosphonates is mediated through the opening of connexion 43 hemichannels and activation of extracellular signal-regulated kinases. The possibility that bisphosphonates used clinically may get access to osteocytes differentially depending on their mineral-binding affinities and inherent structural properties needs to be studied.

**STRUCTURE-ACTIVITY RELATIONSHIPS AND MECHANISM OF ACTION**

The features of the bisphosphonate molecule necessary for biological activity were well defined in the early studies. The P-C-P moiety is responsible for the strong affinity of the bisphosphonates for binding to hydroxyapatite and allows for a number of variations in structure on the basis of substitution in the R1 and R2 positions on the carbon atom (Fig 1). The ability of the bisphosphonates to bind to hydroxyapatite crystals and to prevent both crystal growth and dissolution was enhanced when the R3 side chain (attached to the geminal carbon atom of the P-C-P group) was a hydroxyl group (as in etidronate) rather than a halogen atom such as chlorine (as in clodronate). The presence of a hydroxyl group at the R3 position increases the affinity for calcium (and, thus, bone mineral) because of the ability of bisphosphonates to chelate calcium ions by tridentate rather than bidentate binding.

The ability of bisphosphonates to inhibit bone resorption in vitro and in vivo also requires the P-C-P structure. Monophosphonates (eg, pentane monophosphonate) or P-C-C-P or P-N-P compounds are ineffective as inhibitors of bone resorption. Furthermore, the antiresorptive effect cannot be accounted for simply by adsorption of bisphosphonates to bone mineral and prevention of hydroxyapatite dissolution. It became clear that
bisphosphonates must inhibit bone resorption by cellular effects on osteoclasts rather than simply by physicochemical mechanisms.

After the successful clinical use of clodronate and etidronate in the 1970s and 1980s, more potent antiresorptive bisphosphonates, which had different R² side chains but in which R¹ was unaltered, were studied. In particular, bisphosphonates containing a basic primary amino-nitrogen atom in an alkyl chain (as in pamidronate and alendronate) were found to be 10- to 100-fold more potent than etidronate and clodronate. Then, in the 1980s, there was a phase in which synthesis of novel compounds took place specifically to determine their possible effects on calcium metabolism, with the result that compounds highly effective as inhibitors of bone resorption were identified and studied.

These compounds, especially those that contain a tertiary amino-nitrogen (such as ibandronate and olpadronate), were even more potent at inhibiting bone resorption. Among this generation of compounds that were synthesized to optimize their antiresorptive effects, the most potent antiresorptive bisphosphonates were those containing a nitrogen atom within a heterocyclic ring (as in risedronate and zoledronate), which are up to 10 000-fold more potent than etidronate in some experimental systems (Fig 2).

The analysis of structure-activity relationships allowed the spatial features of the active pharmacophore to be defined in considerable detail even before the molecular mechanism of action was fully elucidated. For maximal potency, the nitrogen atom in the R² side chain must be a critical distance away from the P-C-P group and in a specific spatial configuration. This principle was used successfully for predicting the features required in the chemical design of new and more active compounds.

Although the structure of the R² side chain is the major determinant of antiresorptive potency, both phosphonate groups are also required for the drugs to be pharmacologically active.
In summary, studies of the relationships between bisphosphonate structure and antiresorptive potency suggested that the ability of bisphosphonates to inhibit bone resorption depend on 2 separate properties of the bisphosphonate molecule. The 2 phosphonate groups, together with a hydroxyl group at the R1 position, impart high affinity for bone mineral and act as a “bone hook,” which allows rapid and efficient targeting of bisphosphonates to bone mineral surfaces. Once localized within bone, the structure and three-dimensional conformation of the R2 side chain (as well as the phosphonate groups in the molecule) determine the biological activity of the molecule and influence the ability of the drugs to interact with specific molecular targets. Our understanding of what these molecular targets might be has become much clearer as a result of recent work.

Over the years there have been many efforts to explain how bisphosphonates work on cells, especially via inhibitory effects on enzymes (eg, by direct or indirect inhibition of the osteoclast proton-pumping H+/H2O1 ATPase, phosphatases, or lysosomal enzymes). Because osteoclasts are highly endocytic, bisphosphonate present in the resorption space is likely to be internalized by endocytosis and thereby affect osteoclasts directly. The uptake of bisphosphonates by osteoclasts in vivo has been confirmed by using radiolabeled and fluorescently labeled alendronate, which was internalized into intracellular vacuoles. After cellular uptake, a characteristic morphologic feature of bisphosphonate-treated osteoclasts is the lack of a ruffled border, the region of invaginated plasma membrane facing the resorption cavity. Bisphosphonates also disrupt the cytoskeleton of the osteoclast. Early explanations for these effects invoked the inhibition of protein kinases or phosphatases that regulate cytoskeletal structure, such as protein tyrosine phosphatases. However, a more likely mechanism by which the cytoskeleton may be affected involves loss of function of small GTPases such as Rho and Rac.

Since the early 1990s there has been a systematic effort by our group and others to elucidate the molecular mechanisms of action of bisphosphonates and we have proposed that bisphosphonates can be classified into at least 2 major groups with different modes of action (Fig 3). The first group comprises the non–nitrogen-containing bisphosphonates that perhaps most closely resemble pyrophosphate, such as clodronate and etidronate, and these can be metabolically incorporated into nonhydrolyzable analogues of adenosine triphosphate (ATP) by reversing the reactions of aminoacyl–transfer RNA synthetases. The resulting metabolites contain the P-C-P moiety in place of the β,γ-phosphate groups of ATP, thus resulting in nonhydrolyzable (AppCp) nucleotides. It is likely that intracellular accumulation of these metabolites within osteoclasts inhibits their function and may cause osteoclast cell death. The AppCp-type metabolites of bisphosphonates are cytotoxic when internalized and cause similar changes in morphology to those observed in clodronate-treated cells, possibly by interference with mitochondrial ATP translocases. Overall, this group of bisphosphonates, therefore, seem to act as prodrugs, being converted to active metabolites after intracellular uptake by osteoclasts in vivo.

In contrast, the second group contains the more potent, nitrogen-containing bisphosphonates such as alendronate, risedronate, and zoledronate. Members of this group interfere with other metabolic reactions, notably in the mevalonate biosynthetic pathway, and affect cellular activity and cell survival by interfering with protein prenylation and, therefore, the signaling functions of key regulatory proteins. These mechanisms have been reviewed in detail elsewhere. The mevalonate pathway is a biosynthetic route responsible for the production of cholesterol, other sterols, and isoprenoid lipids such as isopentenyl diphosphate (also known as isopentenyl pyrophosphate), as well as farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP). FPP and GGPP are required for the posttranslational modification (prenylation) of small GTPases such as Ras, Rab, Rho, and Rac, which are prenylated at a cysteine residue in characteristic C-terminal motifs. Small GTPases are important signaling proteins that regulate a variety of cell processes important for osteoclast function, including cell morphology, cytoskeletal arrangement, membrane ruffling, trafficking of vesicles, and apoptosis. Prenylation is
required for the correct function of these proteins because the lipid prenyl group serves to anchor the proteins in cell membranes and may also participate in protein-protein interactions.70

Many observations point to the importance of the mevalonate pathway for osteoclast function and strengthen the proposal that the nitrogen-containing bisphosphonates inhibit osteoclastic bone resorption predominantly by inhibition of this pathway. These bisphosphonates inhibit the synthesis of mevalonate metabolites including FPP and GGPP, and thereby impair the prenylation of proteins,71 and cause alteration of function of small GTPases. There is a strong structure-activity relationship such that changes to the structure of the nitrogen-containing R3 side chain or to the phosphate groups, which alter antiresorptive potency, also influence the ability to inhibit protein prenylation to a corresponding degree.72 An important verification of the critical importance of this pathway has come from showing that the addition of intermediates of the mevalonate pathway (such as FPP and GGPP) could overcome bisphosphonate-induced apoptosis and other events in many cell systems. Another prediction was that if inhibition of the mevalonate pathway could account for the antiresorptive effects of bisphosphonates, then the statin drugs should also inhibit bone resorption. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, one of the first steps in the mevalonate pathway (such as FPP and GGPP) could overcome bisphosphonate-induced apoptosis and other events in many cell systems. Another prediction was that if inhibition of the mevalonate pathway could account for the antiresorptive effects of bisphosphonates, then the statin drugs should also inhibit bone resorption. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, one of the first steps in the mevalonate pathway. They proved to be even more potent than bisphosphonates at inhibiting osteoclast formation and bone resorption in vitro.73,74 an effect that could also be overcome by the addition of geranylgeraniol (which can be used for protein geranylgeranylation) but not farnesol (which is used for protein farnesylation). Hence, it seems that although nitrogen-containing bisphosphonates can prevent both farnesylation and geranylgeranylation of proteins (probably by inhibiting enzymes required for synthesis of FPP and GGPP), loss of geranylgeranylated proteins in osteoclasts is of greater consequence than loss of farnesylated proteins. This is consistent with the known role of geranylgeranylated proteins such as Rho, Rac, and Rap in processes that are fundamental to osteoclast formation and function (eg, cytoskeletal rearrangement, membrane ruffling, and vesicular trafficking75), and further work has confirmed this, particularly the importance of Rap proteins.

The comparison between bisphosphonates and statins is interesting. The statins are widely used as cholesterol-lowering drugs (they are able to lower cholesterol biosynthesis by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase). Despite several studies, there is no substantial evidence that statins have effects on bone when used clinically, perhaps because they are selectively taken up by the liver rather than bone, which is the converse of the case for bisphosphonates. Therefore, this is an excellent example of how drug specificity is achieved by highly selective tissue targeting.

The exact enzymes of the mevalonate pathway that are inhibited by individual bisphosphonates have been partially elucidated. Several enzymes of the pathway use isoprenoid diphosphates as a substrate (isopentenyl pyrophosphate isomerase, FPP synthase, GGPP synthase, squalene synthase) and, thus, are likely to have similar substrate-binding sites. Thus, if nitrogen-containing bisphosphonates act as substrate analogues of an isoprenoid diphosphate, it is possible that these bisphosphonates will inhibit more than 1 of the enzymes of the mevalonate pathway. Early studies revealed that incadronate and ibandronate, but not other bisphosphonates, are inhibitors of squalene synthase, an enzyme in the mevalonate pathway that is required for cholesterol biosynthesis.76,77 Inhibition of squalene synthase, however, would not lead to inhibition of protein prenylation.

However, it is now clear that farnesyl pyrophosphate synthase (FPFS) is a major site of action of the nitrogen-containing bisphosphonates (N-bisphosphonates).78 FPFS catalyzes the successive condensation of isopentenyl pyrophosphate with dimethylallyl pyrophosphate and geranyl pyrophosphate. There is a strong relationship among individual bisphosphonates between inhibition of bone resorption and inhibition of FPFS, with the most potent bisphosphonates having IC50 values (concentration that inhibits response by 50%) in the nanomolar range.79 Modeling studies have provided a molecular rationale for bisphosphonate binding to FPFS.80 Our recent studies using protein crystallography, enzyme kinetics, and isothermal titration calorimetry led to the first published high-resolution radiograph structures of the human enzyme in complexes with risedronate and zoledronate.81 These agents bind to the dimethylallyl/geranyl pyrophosphate ligand pocket and induce a conformational change. The interactions of the N-bisphosphonate cyclic nitrogen with Thr201 and Lys200 suggest that these inhibitors achieve potency by positioning their nitrogen in a proposed carbocation binding site. This explains how the nitrogen moiety is so important to the potency of these bisphosphonates. Kinetic analyses reveal that inhibition is competitive with geranyl pyrophosphate and is of a slow, tight-binding character, which indicates that isomerization of an initial enzyme-inhibitor complex occurs after binding of the N-bisphosphonate.

Taken together, these observations clearly indicate that bisphosphonates can be grouped into 2 classes: those that can be metabolized into nonhydrolyzable analogues of ATP (the least potent bisphosphonates) and those that are not metabolized but can inhibit protein prenylation (the potent, nitrogen-containing bisphosphonates). The identification of 2 such classes may help to explain some of the other pharmacologic differences between the 2 classes.
CLINICAL APPLICATIONS OF BISPHOSPHONATES

After it was shown that bisphosphonates inhibited experimentally induced calcification and bone resorption, their potential application to clinical disorders was obvious, but it took many years for them to become well established.

The earliest clinical applications of bisphosphonates included use of etidronate as an inhibitor of calcification in fibro dysplasia ossificans progressiva (formerly known as myositis ossificans) and in patients who had undergone total hip replacement surgery to prevent subsequent heterotopic ossification and improve mobility.83

One of the other early clinical uses of bisphosphonates was as agents for bone imaging, “bone scanning,” for which they still remain outstandingly useful for detecting bone metastases and other bone lesions. The application of pyrophosphate and simple bisphosphonates as bone-scanning agents depends on their strong affinity for bone mineral, particularly at sites of increased bone turnover, and their ability to be linked to a γ-emitting technetium isotope.84,85

The most impressive clinical application of bisphosphonates has been as inhibitors of bone resorption, especially for diseases in which no effective treatment existed previously. Thus, bisphosphonates became the treatment of choice for a variety of bone diseases in which excessive osteoclast activity is an important pathologic feature, including Paget disease of bone, metastatic and osteolytic bone disease, and hypercalcemia of malignancy, as well as osteoporosis.

The clinical pharmacology of bisphosphonates is characterized by low intestinal absorption (~1%–4%) but highly selective localization and retention in bone. Significant adverse effects of bisphosphonates are minimal.86–88 Although there are more similarities than differences between individual compounds and each bisphosphonate is potentially capable of treating any of the disorders of bone resorption in which they are used, in practice different compounds have come to be favored for the treatment of different diseases. There are currently at least 10 bisphosphonates (etidronate, clodronate, tiludronate, pamidronate, alendronate, risedronate, zoledronate, and ibandronate and, to a limited extent, olpadronate and neridronate) that have been registered for various clinical applications in various countries. To a major extent, the diseases in which they are used reflects the history of their clinical development and the degree of commercial interest in and sponsorship of the relevant clinical trials.

Paget disease was the first clinical disorder in which a dose-dependent inhibition of bone resorption could be demonstrated by using bisphosphonates in humans.89,90 Bisphosphonates have become the most important drugs used in the treatment of Paget disease.91 For many years pamidronate given by intravenous infusion was used extensively,92 but the newer and more potent bisphosphonates can produce even more profound suppression of disease activity than was possible with the bisphosphonates available in previous years.93,94 The latest advance is with zoledronate,95 which, when given as a single 5-mg infusion, produced a greater and longer-lasting suppression of excess bone turnover than even oral risedronate given at 30 mg/day over 2 months, hitherto one of the most effective treatments.

In terms of commercial success, the use of bisphosphonates in oncology has been preeminent. Many cancers in humans are associated with hypercalcemia (raised blood calcium) and/or increased bone destruction. Bisphosphonates are remarkably effective in the treatment of bone problems associated with malignancy96 and are now the drugs of choice.97–99 Clinical trials that investigate the benefit of bisphosphonate therapy use a composite end point defined as a skeletal-related or bone event, which typically includes pathologic fracture, spinal cord compression, radiation or surgery to bone, and hypercalcemia of malignancy. Bisphosphonates significantly reduce the incidence of these events in myeloma100 and in patients with breast cancer metastases101,102 and in metastatic prostate cancer,103 lung cancer, renal cell carcinoma, and other solid tumors. The goals of treatment for bone metastases are also to prevent disease-related skeletal complications, palliate pain, and maintain quality of life. Zoledronate,104 pamidronate, clodronate, and ibandronate105,106 have demonstrated efficacy compared with placebo.

There is the important possibility that the survival of patients may be prolonged107–109 in some groups of patients. Recently, osteonecrosis of the jaw110 was identified as a potential complication of high-dose bisphosphonate therapy in malignant diseases.

The other area of outstanding commercial success with bisphosphonates has been in the therapy of osteoporosis, which is a major public health problem.111,112 Up until the 1990s, there were few treatments for osteoporosis. As a drug class the bisphosphonates have emerged in the past few years as the leading effective treatments for postmenopausal and other forms of osteoporosis. Etidronate was the first of these,113–115 followed by alendronate116–118 and then risedronate.119,120 All have been approved as therapies in many countries and can increase bone mass and reduce fracture rates at the spine by 30% to 50% and at other sites in postmenopausal women.121 The reduction in fractures may be related not only to the increase in bone mass arising from the inhibition of bone resorption and reduced activation frequency of bone-remodeling units but also to enhanced osteon mineralization.122 These bisphosphonates also prevent bone loss associated with glucocorticosteroid administration.123,124

Among the newer bisphosphonates, ibandronate125 was introduced recently as a once-monthly tablet. In addition to formulations to be taken by mouth weekly or
monthly, new routes of administration are being studied, especially periodic (eg, 3 monthly) injections with ibandronate and once-yearly treatment with zoledronate.126 This has the attraction of delivering a defined dose without the variability associated with oral administration as well as avoiding potential gastrointestinal intolerance. If these approaches are accompanied by greater compliance and convenience, they are likely to become popular methods of treatment.

Other clinical issues under consideration with bisphosphonates include the choice of therapeutic regimens (eg, the use of intermittent dosing rather than continuous, intravenous versus oral therapy), the optimal duration of therapy, the combination with other drugs such as teriparatide, and their extended use in related indications (eg, glucocorticosteroid-associated osteoporosis, male osteoporosis, childhood osteopenic disorders, arthritis, and other disorders). Therefore, there is much that needs to be done to improve the way in which existing drugs can be used and to introduce new ones.

In pediatrics, pamidronate has proved remarkably effective in increasing bone in children with the inherited “brittle-bone” disorder, osteogenesis imperfecta.127,128

**SOME CURRENT ISSUES WITH BISPHOSPHONATES: BONE ARCHITECTURE, STRUCTURE, AND STRENGTH, AND ON BONE HEALING AND FRACTURE REPAIR**

Many experimental and clinical studies show that bisphosphonates conserve bone architecture and strength.129–133 However, there have been concerns about whether the use of prolonged high doses of bisphosphonates may impair bone turnover to such an extent that bone strength is impaired. High doses in animals are associated with increased microdamage134,135 and even fractures.136 It has been suggested that bisphosphonates might prevent naturally occurring microscopic cracks in bone from healing. There have been isolated reports of adynamic bone associated with bisphosphonate usage.137 but long-term use of the bisphosphonates in the therapy of osteoporosis seems to be safe.138 Case reports of induction of osteoporosis-like lesions in children who were treated with excessive doses of pamidronate have been published.139

A question often asked is whether bisphosphonates inhibit fracture repair. By reducing bone turnover one might expect bisphosphonates to interfere with fracture healing. However, a recent long-term study in a beagle dog model that simulated fracture repair has demonstrated that ibandronate treatment did not adversely affect normal bone healing.140 Studies of repair processes after creating drill-hole defects in dogs also showed no impairment with ibandronate.141

Several other recent studies raised the intriguing possibility that bisphosphonates may enhance fracture repair and related processes.142 In studies of the osseointegration of metal implants in ovariectomized rats, treatment with ibandronate resulted in improved osseointegration rather than impairment of the healing process.143 Potential applications of bisphosphonates in orthopedics include protection against loosening of prostheses,144 better integration of biomaterials and implants, improved healing in distraction osteogenesis,145 and conserving bone architecture after osteonecrosis146,147 and in Perthes disease.148

There are potentially important differences between clinically useful bisphosphonates regarding their potency and duration of action. Efficacy is closely related to affinity for bone mineral and ability to inhibit FPP synthase. Recent studies showing that there are marked differences among bisphosphonates in binding to hydroxyapatite149 may explain the variations in retention and persistence of effect that have been observed in animal and clinical studies. In the case of zoledronic acid, in particular, the remarkable magnitude of effect and prolonged duration of action can be explained in part by these new observations. In explaining the long duration of action, it has been proposed that there is continual

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**FIGURE 4**

Bisphosphonate (BP) uptake and detachment from bone surfaces: effect of binding affinity on recirculation of bisphosphonate on and off bone surfaces. The differences in mineral-binding affinity may affect distribution into different bone compartments and persistence of drug action at bone surfaces. (Adapted from Nancollas GH, Tang R, Phipps RJ, et al. Bone. 2006;38:617–627.)
recycling of bisphosphonate off and back onto the bone surface. This notion is supported by observations that bisphosphonates can be found in plasma and urine many months after dosing (Fig 4).

There are numerous examples of bisphosphonates having effects on cells and tissues outside the skeleton. The effects on osteoclast precursors, tumor cells, macrophages, and γ,δ-T cells are examples and in all cases are probably explained by sufficient bisphosphonates entering cells to inhibit the mevalonate pathway. A well-recognized adverse effect of the nitrogen-containing bisphosphonates is that they cause an acute-phase response in vivo,150,151 which can lead to induction of fever and “flu-like” symptoms in patients. These effects are transient and occur predominantly on first exposure to the drug, especially with intravenous administration. The mechanism has been attributed to release of proinflammatory cytokines, and the mechanism has been further unraveled by showing that it involves selective receptor-mediated activation of γ,δ-T cells, leading to their proliferation and activation.152 The bisphosphonate effect involves the mevalonate pathway in vitro and can be overcome by using statins.153

Another interesting aspect of these nonskeletal effects are the observations made on protozoan parasites, the growth of which can be inhibited by bisphosphonates acting on FPPS.154,155 In the future, other clinical indications ripe for future study include the prevention of bone loss and erosions in rheumatoid arthritis, possible applications in other joint diseases, the reduction of bone loss associated with periodontal disease, and loosening of joint prostheses.

The recent elucidation of the likely mode of action of bisphosphonates within cells opens up the possibility of exploiting the subtle and potentially important differences between the classes of bisphosphonates and individual compounds.

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Experience With Bisphosphonates in Osteogenesis Imperfecta

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The author has indicated he has no financial relationships relevant to this article to disclose.

ABSTRACT

Until recently, medical management of osteogenesis imperfecta, a genetic disorder of reduced bone mass and frequent fractures, was elusive, and treatment was focused on maximizing mobility and function. The introduction of bisphosphonates for the treatment of osteogenesis imperfecta 14 years ago changed this paradigm. Cyclic intravenous pamidronate therapy leads to an increase in bone density and a decrease in fracture rate in patients with osteogenesis imperfecta. Pamidronate therapy has a positive impact on functional parameters including improved energy, decreased bone pain, and increased ambulation. Histomorphometric studies have shown that the reduced osteoclast activity results in gains in cortical thickness and trabecular bone volume. Potential negative effects may include prolonged time to heal after osteotomies and a decrease in the rate of bone remodeling. Overall, it seems clear that the benefits of pamidronate therapy outweigh its potential risks in moderate-to-severe osteogenesis imperfecta, and pamidronate therapy has become the standard of care for patients with this condition. Questions remain regarding when treatment should be stopped and the need for pamidronate therapy in patients with mild osteogenesis imperfecta.
OSTEOGENESIS IMPERFECTA (OI) is a heritable disorder that is characterized by bone fragility and reduced bone mass. Severity varies widely, ranging from a lethal form with intrauterine fractures to a very mild form with no or few fractures and normal growth. Excessive manifestations include blue sclera, dentinogenesis imperfecta, skin and ligament hyperlaxity, and presence of wormian bones within cranial sutures. Most patients with a clinical diagnosis of OI harbor a mutation in 1 of the 2 genes encoding the α chains of type I collagen. There is growing interest in the search for other genes that may cause the bone abnormalities in patients with OI in whom no mutation in either COL1A1 or COL1A2 can be found.

Until about 10 years ago, medical management of OI consisted mainly of rehabilitation, physiotherapy, and corrective surgery. The overall aim was for each patient to reach his or her potential in terms of mobility and functional capabilities. Various forms of medical therapy to enhance bone formation have been attempted (vitamin D, fluoride, calcitonin, etc) with no tangible results. More promising data have been obtained by using bisphosphonates, which are potent antiresorptive agents. The rationale for using such drugs was found in our histomorphometric studies, which showed a high bone turnover rate in patients with OI and the frequent occurrence of superimposed disuse bone loss caused by impaired ambulation attributed to frequent fractures, deformities, and chronic pain.

At the Shriners Hospital for Children in Montreal, the bisphosphonate program was started in October 1992 and uses mostly cyclic intravenous pamidronate. Up to now, 233 patients with moderate-to-severe OI have been treated for periods up to 7 years. The drug has been given in 3-day cycles, every 2 to 4 months depending of the age of the patients (the younger the patient, the shorter the interval between cycles). In all instances, the annual dose of pamidronate was 9 mg/kg per year. Within 1 to 2 weeks after the first infusion cycle, bone pain decreased considerably and often disappeared completely. The patients also felt more energetic, as evidenced by a significant increase in grip force. Bone mineral density steadily increased over time in the lumbar spine. When the data were transformed to take into account the increase in the third dimension resulting from growth (volumetric bone mineral density), the gain was still evident (>75% over 4 years). It was accompanied by a change in shape and size of vertebral bodies (L1–L4). On lateral views, compressed vertebrae became larger and more rectangular, an effect of the drug amplified by the growth process. Fracture incidence decreased from 2.3 to 0.6 events per year in our first report. In infants under 2 years of age with severe OI, fracture incidence was 2.6 events per year compared with 6.3 events per year in untreated controls. This positive effect of therapy has been confirmed by several other studies. One should keep in mind, however, that such results are directly influenced by age, severity of OI, degree of ambulation, and social environment. In other words, treatment success may translate into higher risk of fractures. In a recent trial, using a daily dosage of oral alendronate, such a beneficial effect on fracture incidence could not be demonstrated. Pamidronate therapy, when started early in life, also has a positive effect on the degree of ambulation. When assessed with both Pediatric Evaluation of Disability Inventory scores and a modified Bleck mobility scale, the effect was significantly evident.

Because osteoclasts play an important role in the process of endochondral bone formation, it was feared that long-term administration of bisphosphonates in growing individuals could have a negative impact on longitudinal growth. This turned out not to be the case. In 41 subjects treated for at least 4 years, we observed, in fact, a significant height gain. Another major benefit, the gain in bone mass, was demonstrated in bone histomorphometric studies. After 2.4 years of treatment in 45 patients, there was an 88% gain in cortical thickness and a 46% gain in trabecular bone volume, which can be explained by the drug reducing osteoclast activity and, thus, the rate of endocortical resorption. Because periosteal new bone apposition continues during the modeling process, the net effect is a gain in cortical bone mass. In the metaphyseal areas, the gain in bone volume was a result of survival of a larger number of calcified cartilage spicules, which serve as scaffolds for new bone deposition. The thickness of individual trabeculae was not changed. An observation frequently made in patients receiving cyclic pamidronate is the occurrence of dense metaphyseal lines parallel to the growth plate. Each line is the signature of a treatment cycle. It is made in part of unresorbed calcified cartilage (~25% in the line nearest to the plate) and calcified bone. These transverse trabeculae may improve bone mechanical resistance. As they move away from the plate, they are progressively remodeled. The distance between lines reflects the amount of bone formed during the intervals between treatment cycles and, thus, are a measure of the elongation process under individual growth plates. The major, potentially negative, adverse effect of long-term bisphosphonate administration is a rapid and important reduction in bone turnover rate. Its consequences have yet to be fully evaluated, but they may include prolonged healing time after osteotomies but not fractures and delayed removal of damaged bone matrix. In adults, this slowdown of remodeling activity with long-term use of bisphosphonates has been considered as an advantage because it allows for more complete mineralization of the bone matrix to improve its mechanical resistance. This advantage cannot be extrapolated to bone in patients with OI. Indeed, we demonstrated that, before any treatment, bone in patients with OI showed higher av-
verage mineralization density than normal bone. This may be the result of failure in matrix assembly such that it has a higher water-volume fraction available for mineral deposition. This is not significantly altered by subsequent pamidronate treatment. Thus, pamidronate increases the amount of bone but not its material density. Assessment of the biomechanical properties of bone material measured by nanoindentation confirm that bone in patients with OI is harder than normal bone at the material level but is not altered by pamidronate.

In conclusion, treatment with cyclical intravenous pamidronate has changed the face of moderate-to-severe OI. Over up to 7 years of treatment, the following positive effects have been documented:

- good short-term safety (particularly with regard to renal function);
- suppression or significant reduction in chronic bone pain;
- gain in muscle force;
- increase in density and size of vertebral bodies;
- thickening of bone cortex; and
- gain in growth rate.

Some negative effects have also been observed:

- decrease in bone remodeling rate;
- reduction in growth plate cartilage resorption; and
- delay in the healing of osteotomy sites.

Bisphosphonates stay in bone for a very long time. They can be released during remodeling. Whether this would cause problems, for instance, during pregnancy remain unclear. Thus, at this stage, it is prudent to restrict this therapeutic approach to moderate-to-severe cases of OI in which the potential benefits clearly outweigh the risks. No established benefits have been documented in the mild cases. How long a patient should be treated, what the criteria for stopping treatment are, and what the criteria are for reactivating it at a later stage remain open questions.

ACKNOWLEDGMENT
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Glucocorticoid-Induced Osteoporosis in Children: Impact of the Underlying Disease

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ABSTRACT

Glucocorticoids inhibit osteoblasts through multiple mechanisms, which results in significant reductions in bone formation. The growing skeleton may be especially vulnerable to adverse glucocorticoid effects on bone formation, which could possibly compromise trabecular and cortical bone accretion. Although decreased bone mineral density has been described in various pediatric disorders that require glucocorticoids, and a population-based study reported increased fracture risk in children who required >4 courses of glucocorticoids, some of the detrimental bone effects attributed to glucocorticoids may be caused by the underlying inflammatory disease. For example, inflammatory cytokines that are elevated in chronic disease, such as tumor necrosis factor α, suppress bone formation and promote bone resorption through mechanisms similar to glucocorticoid-induced osteoporosis. Summarized in this review are changes in bone density and dimensions during growth, the effects of glucocorticoids and cytokines on bone cells, the potential confounding effects of the underlying inflammatory-disease process, and the challenges in interpreting dual-energy x-ray absorptiometry results in children with altered growth and development in the setting of glucocorticoid therapy. Two recent studies of children treated with chronic glucocorticoids highlight the differences in the effect of underlying disease, as well as the importance of associated alterations in growth and development.

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Key Words
- glucocorticoids, bone mineral density, bone mineral content, glucocorticoid-induced osteoporosis, growth

Abbreviations
- GIO—glucocorticoid-induced osteoporosis
- BMD—bone mineral density
- TNF-α—tumor necrosis factor α
- DXA—dual-energy x-ray absorptiometry
- QCT—quantitative computed tomography
- BMC—bone mineral content
- RANKL—receptor activator of nuclear factor κB ligand
- IL—interleukin
- SSNS—steroid-sensitive nephrotic syndrome
- CI—confidence interval

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PEDiatrics (ISSN Numbers: Print, 0031-4005; Online, 1098-4275); published in the public domain by the American Academy of Pediatrics
During childhood and adolescence, skeletal development is characterized by gender-, maturation-, and race-specific increases in cortical dimensions and trabecular density. The 2000 National Institutes of Health Osteoporosis Prevention, Diagnosis, and Therapy Consensus Development Conference identified bone accrual during childhood as a critical determinant of lifelong skeletal health. The authors of the resulting consensus statement specifically called for research to determine the impact of chronic diseases and glucocorticoid therapy on bone accrual in children.

Glucocorticoid-induced osteoporosis (GIO) is the most common cause of secondary osteoporosis in adults and is the result of profound effects of glucocorticoids on bone cells. Glucocorticoids inhibit osteoblastogenesis and promote osteoblast apoptosis, which results in significant reductions in bone formation. Studies in adults have demonstrated that glucocorticoids cause rapid, dose-dependent bone loss and increased fracture risk. The growing skeleton may be especially vulnerable to adverse glucocorticoid effects on bone formation, which could possibly compromise trabecular and cortical bone accretion.

Glucocorticoids are used in myriad pediatric diseases. Decreased bone mineral density (BMD) has been described in various pediatric disorders that require glucocorticoids, including asthma, juvenile rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, and organ transplantation. A population-based study reported increased fracture risk in children who required ≥4 courses of glucocorticoids. Although these studies demonstrate a correlation between glucocorticoids, bone deficits, and fracture risk, some of the detrimental bone effects attributed to glucocorticoids may be caused by the underlying inflammatory disease. For example, inflammatory cytokines, such as tumor necrosis factor α (TNF-α), suppress bone formation, promote bone resorption, and are increased in rheumatoid arthritis, inflammatory bowel disease, and systemic lupus erythematosus. Furthermore, alterations in growth related to glucocorticoids and the underlying disease may confound dual-energy x-ray absorptiometry (DXA) measures of BMD.

Summarized in this review are changes in bone density and dimensions during growth, the effects of glucocorticoids and cytokines on bone cells, the potential confounding effects of the underlying inflammatory-disease process, and the challenges in interpreting DXA results in children with altered growth and development in the setting of glucocorticoid therapy. Two recent studies of children who were treated chronically with glucocorticoids highlight the differences in the effect of underlying disease, as well as the importance of concurrent alterations in body composition.

Bone Modeling and Remodeling

The shape and structure of bones are continuously modified and renovated by the 2 processes of modeling and remodeling, both of which result in the replacement of old bone with new bone. Remodeling is the major process in adults and does not result in a change of the bone shape. Remodeling takes place in the basic bone multicellular units on the trabecular surface and within the cortical bone. Normally, bone resorption by osteoclasts is followed by bone formation by osteoblasts; teams of osteoclasts and osteoblasts are juxtaposed in the bone multicellular units, and bone resorption and formation are coupled. In the young-adult skeleton, the newly formed bone completely replaces the bone lost in the resorption phase. This process is vital for repairing microdamage and maintaining skeletal integrity. After midadulthood, the amount of resorption slightly exceeds formation, which results in a negative bone balance with aging.

In contrast, modeling results in new bone formed at a location different from the site of bone resorption. This process results in an increase in bone diameter and modification of bone shape. Figure 1 summarizes the complex interplay of site-specific bone-resorption and -formation activities that are necessary to achieve bone growth from length A to B. Growth in the diameter of the cortical shaft is the result of bone formation at the
outer (periosteal) surface and bone resorption at the inner (endosteal) surface. Simultaneously, the growth plate moves upward and the wider parts of the bone must be reshaped into a diaphysis. This reshaping is accomplished by continuous resorption by osteoclasts beneath the periosteum. The impact of glucocorticoid therapy on bone modeling and structure has not been well characterized.

Trabecular BMD, as measured by quantitative computed tomography (QCT), does not increase before puberty.12,13 During puberty, trabecular BMD increases significantly as a result of increases in trabecular thickness.14 The pubertal increases in BMD are comparable in girls and boys but are significantly greater in black compared with white adolescents.12,13,15 Studies of cortical BMD and dimensions in the appendicular skeleton produced conflicting results, potentially because of differences between the upper and lower extremities and in measurement techniques. Most studies have reported significant increases in cortical BMD with age (reviewed by Hogler et al16). An early study of cortical dimensions, based on two-dimensional radiogrammetry, concluded that the greater cortical bone mass in male subjects was attributable to gender differences in the rate of endosteal apposition and resorption.17 Subsequent peripheral QCT studies in the upper extremities also suggested gender differences in the endocortical surface, with constant dimensions with age in female subjects and increasing dimensions with age in male subjects.18 Furthermore, later age at menarche in girls is associated with greater endosteal dimensions in adulthood.19 In contrast, studies in the weight-bearing femur15,16 and tibia20 failed to demonstrate gender differences in endocortical resorption. Given the distinct effects of puberty and gender on trabecular and cortical bone, the structural implications of glucocorticoid therapy may differ in pubertal and prepubertal children.

FUNCTIONAL MUSCLE-BONE UNIT

According to Wolff’s law, bone grows in response to the magnitude and direction of the forces to which it is subjected.21 This response keeps mechanically induced deformation of bone (strain) at a set point. Bone deformation induces canalicular fluid flow that is detected by osteocytes.22 Osteocytes transduce signals that induce an anabolic response to increase bone strength.23 This capacity of bone to respond to mechanical loading with increased bone strength is greatest during growth;24 mechanical signals that are osteogenic in the young skeleton fail to stimulate bone formation in the mature skeleton.25 Hormones and nutrients influence mechanical loads by influencing linear growth and muscle mass and may alter the muscle-bone set point.26

The observed strong correlation between muscle mass and bone mass has prompted numerous investigators to advocate a multistage algorithm for the assessment of bone measures relative to muscle mass in children. Proposed strategies include assessing bone mineral content (BMC) relative to body weight or lean mass and varied multistage models for BMC that incorporate age, ethnicity, height, weight, bone area, and pubertal stage.27–32

Glucocorticoids are also well known to cause muscle wasting.33 Muscle weakness results from muscle atrophy because of accelerated catabolism of muscle proteins. Therefore, glucocorticoid-induced myopathy may contribute to bone deficits via the functional muscle-bone unit.

GLUCOCORTICOID EFFECTS ON BONE FORMATION AND RESORPTION

Decreased bone formation is the primary mechanism for bone loss in GIO.2 Mesenchymal stems cells, which also give rise to adipocytes, myoblasts, and chondrocytes, differentiate into osteoblasts. Glucocorticoids shift the cellular differentiation away from osteoblasts and toward adipocytes and prevent the termination differentiation of osteoblasts.34 Osteoblast numbers are decreased further by glucocorticoid-induced increases in osteoblast apoptosis.3 In addition, glucocorticoids inhibit osteoblast production of bone-matrix components.35 Finally, glucocorticoids suppress the synthesis of insulin-like growth factor I, an agent that enhances bone formation.36 The cellular response to glucocorticoids also includes an early phase of increased bone resorption, which is probably a result of the increased expression of receptor activator of nuclear factor κB ligand (RANKL) and decreased expression of osteoprotegerin; increased RANKL and decreased osteoprotegerin both promote osteoclastogenesis, as detailed below.37,38 However, typically, a more chronic state of decreased bone resorption develops as a result of loss of cell signaling to osteoclast progenitors.39 Decreased bone formation and resorption have also been observed in the setting of increased endogenous glucocorticoid production (eg, in burn injury in children40). The effects of glucocorticoids on bone cells are summarized in Table 1.

Patients treated with glucocorticoids have an underlying disease that frequently also carries a risk of osteoporosis. Therefore, the independent effects of glucocorticoids on bone turnover and bone structure during growth are not readily apparent from clinical studies. However, recent animal models demonstrated that glu-

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<th>TABLE 1</th>
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<td>Shift in cellular differentiation of stem cells away from osteoblasts</td>
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<td>Inhibition of osteoblast production of bone matrix</td>
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<td>Decrease in synthesis of insulin-like growth factor I</td>
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<td>Transient increase in bone resorption</td>
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<td>Promotion of osteoclastogenesis by increasing RANKL and decreasing osteoprotegerin expression in stromal and osteoblastic cells</td>
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corticocorticoid administration during growth resulting in decreased bone formation, decreased bone resorption, reductions in the age-dependent increases in trabecular bone mineral and trabecular thickness, and reductions in linear growth and accrual of cortical thickness in the femur. These deficits were associated with decreased bone strength in the vertebral and femur in mechanical testing. It is notable that it is unclear if the reductions in femoral cortical thickness were proportionate to the significant reductions in bone length; that is, did the bones have normal cortical thickness and strength relative to the shorter length?

OSTEOIMMUNOLOGY: THE INTERPLAY BETWEEN THE IMMUNE SYSTEM AND BONE

Three groups of cytokines are particularly important in bone physiology: interleukin 6 (IL-6), TNF-α, and IL-1. The effects of TNF-α on bone formation are strikingly similar to the effects of glucocorticoids. TNF-α inhibits osteoblast differentiation, inhibits osteoblast synthesis of collagen, and promotes osteoblast apoptosis. The effects of selected inflammatory cytokines on bone cells are summarized in Table 2.

RANKL stimulates osteoclast differentiation and activation and inhibits osteoclast apoptosis. In contrast, osteoprotegerin acts as a decoy receptor for RANKL and acts as an inhibitor of bone resorption. TNF-α, IL-1, and IL-6 exert their osteoclastogenic effects through increased RANKL, as well as through other autocrine and paracrine pathways that are independent of RANKL. It is notable that some studies of bone resorption markers in inflammatory conditions have shown increased resorption, whereas another study demonstrated decreased resorption in the setting of increased endogenous cortisol. It has been hypothesized that the number of osteoblasts are decreased to the point that RANKL production is reduced.

In addition to detrimental effects on bone metabolism, inflammatory cytokines such as TNF-α and IL-1 also adversely affect whole-body protein and energy metabolism, which is similar to glucocorticoid effects. Specifically, TNF-α-induced activation of nuclear factor κB inhibits skeletal-muscle differentiation by suppressing MyoD messenger RNA. Therefore, cytokine-induced reductions in muscle mass may contribute to bone deficits in chronic inflammatory conditions through the functional bone-muscle unit described above.

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<th>TABLE 2</th>
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<td>TNF-α inhibits osteoblast differentiation</td>
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<td>TNF-α inhibits collagen synthesis in osteoblasts</td>
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<td>TNF-α promotes osteoblast apoptosis</td>
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<td>Increase in bone resorption</td>
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<td>IL-1, TNF-α, and IL-6 increase RANKL and promote osteoclastogenesis</td>
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Infliximab, a chimeric monoclonal antibody to TNF-α, is effective in both inducing and maintaining clinical remission in patients with refractory Crohn disease and in patients with inflammatory arthropathies. Recent observational studies of adults who were treated with infliximab highlight the importance of TNF-α in the bone and muscle deficits observed in these diseases; infliximab therapy was associated with significant increases in markers of bone formation, decreases in markers of bone resorption, increases in BMD, and increases in lean mass (without changes in fat mass).

LIMITATIONS OF DXA IN THE ASSESSMENT OF GIO IN CHILDREN

DXA is, by far, the most widely used technique for measuring bone mass in children. However, DXA is a two-dimensional technique in which bone is presented as the combined sum of cortical and trabecular bone within the projected bone area, concealing the distinct structural characteristics. DXA provides an estimate of BMC per anatomic region; dividing the BMC (in grams) by the projected area of the bone (in centimeters squared) then derives areal BMD (in grams per centimeters squared). This BMD is not a measure of volumetric density (in grams per centimeters cubed) because it provides no information about bone depth. Bones of larger width and height are thicker. Because bone thickness is not factored into DXA results, reliance on areal BMD systematically underestimates bone density in shorter people. In evaluating children who receive glucocorticoid therapy, one could falsely attribute the decreased areal BMD for age as evidence for osteopenia rather than a glucocorticoid-induced reduction in height for age.

Two recent studies illustrated that failure to consider the confounding effect of height results in an overestimation of bone deficits in children with chronic disease. First, Wren et al compared DXA areal BMD and QCT volumetric BMD z scores in the spine of 200 healthy children and 200 chronically ill children. The hypothesis of the study was that DXA results in the overdiagnosis of osteoporosis (defined as a z score of less than −2.0) in children with poor growth. Consistent with this hypothesis, a significantly greater proportion of children were classified as osteopenic according to DXA (76 of 400) compared with QCT (25 of 400), particularly among children below the 5th percentile for height and/or weight for age. Using QCT as the standard for this comparison, the specificity of a DXA z score less than −2.0 was 94% among healthy children but only 74% among the chronically ill children; that is, among the 179 ill children with QCT z scores greater than −2.0, 47 (26%) had DXA z scores less than −2.0. Second, Gafni and Baron reported that inattention to the confounding effect of short stature resulted in inappropriate referral for possible inclusion in a childhood osteoporosis protocol on the basis of low DXA-derived spine areal BMD.
TWO MODELS OF GIO IN CHILDREN: CROHN DISEASE AND NEPHROTIC SYNDROME

The following summary considers 2 studies recently reported by our group of children who were receiving glucocorticoids chronically for Crohn disease or steroid-sensitive nephrotic syndrome (SSNS). Crohn disease was associated with significant reductions in BMI and whole-body BMC. In contrast, SSNS was associated with greater BMI, normal spine BMC, and greater whole-body BMC compared with controls. Fig 2 illustrates whole-body BMC relative to height in the subjects with Crohn disease (Fig 2A) and SSNS (Fig 2B), each compared with healthy controls. Differences in the characteristics of the underlying disease are considered as an explanation for these different patterns of bone health in children treated with chronic glucocorticoids. Multivariate analyses are presented to address the confounding effects of growth and maturation.

Crohn Disease

Crohn disease is an idiopathic, lifelong, destructive chronic inflammatory condition of the gastrointestinal tract. The pathogenesis has been linked to genetic and environmental factors that lead to sustained activation of the mucosal immune response. Disease rates are highest in Westernized countries, and the incidence rate in children is increasing. The incidence of pediatric Crohn disease is ~7 new cases per 100 000 children per year. In addition to the usual symptoms of diarrhea, abdominal pain, weight loss, anemia, and rectal bleeding, children may exhibit growth failure years before disease diagnosis. Anorexia, malabsorption, and increased metabolic demands all contribute to poor growth. Small bowel disease may impair absorption of iron, zinc, folate, and vitamin B₁₂. High-dose glucocorticoids are widely used in the treatment of Crohn disease.

Osteopenia has been well documented in children and adults with inflammatory bowel disease. Six children with spine fractures were identified at our institution, and hip, spine, and forearm fractures are significantly increased in adults with Crohn disease. Cellular inflammatory pathways in Crohn disease activate the protean transcriptional regulatory factor nuclear factor κB with increased production of a variety of cytokines such as IL-6 and TNF-α. Serum from children with Crohn disease impairs osteoblast function and differentiation in vitro.

We recently reported significant bone and muscle deficits in a cross-sectional study of children and young adults with established Crohn disease. Whole-body BMC, lean mass, and fat mass were assessed by DXA in 104 subjects with Crohn disease and 233 healthy controls, 4 to 26 years of age. Individuals with Crohn disease had significantly lower height-for-age, BMI-for-age, and whole-body lean-mass-for-height z scores than healthy controls (all P < .001). Ninety percent of subjects with Crohn disease had been treated with glucocorticoids. The cumulative exposure averaged 7900 mg over 15.2 months, which resulted in an average dose of 0.50 mg/kg per day.

The least-adjusted models assessed whole-body BMC in subjects with Crohn disease compared with controls, adjusted for age and race, and revealed substantial deficits (Table 3). Assessment of BMC without consideration of the decreased skeletal size for age in subjects with Crohn disease may overestimate bone deficits. Accordingly, the second model was also adjusted for height. Adjustment for height attenuated the Crohn disease effect; however, significant BMC deficits persisted in male and female subjects with Crohn disease compared with controls. To determine if delayed pubertal maturation for age contributed to the decreased BMC in those with Crohn disease, the third model included Tanner stage. Adjustment for delayed pubertal maturation did not appreciably change the estimate of BMC deficits in the subjects with Crohn disease. The fourth and final
None of the glucocorticoid measures were significantly correlated with BMC-for-height z scores. However, height z score was negatively and significantly associated with duration of glucocorticoid therapy (r = −0.24; P = .02) and cumulative (milligrams per kilogram) glucocorticoid dose (r = −0.36; P < .001). Parenteral nutrition, isolated upper tract disease, hypoalbuminemia, nasogastric feeding, and decreased BMI z scores were associated with decreased BMC-for-height z scores.

A subsequent analysis quantified lean and fat mass in these same subjects with Crohn disease, relative to height and pubertal maturation, compared with healthy controls. Although Crohn disease was associated with significant deficits in lean mass, adjusted for height, age, race, and Tanner stage (P = .003), fat mass was not decreased (mean fat-mass-for-height z score = −0.04 ± 0.86). Within the controls, fat mass for height was positively associated with lean mass for height (r = 0.41; P < .0001); this association was absent in those with Crohn disease. Therefore, subjects with Crohn disease exhibited significant deficits in lean mass with preserved fat mass, which is consistent with inflammatory cachexia.

Steroid-Sensitive Nephrotic Syndrome
In contrast with Crohn Disease, childhood SSNS syndrome provides a clinical model of chronic glucocorticoid therapy in the absence of significant underlying disease activity. The nephrotic state is clinically quiescent as long as high-dose glucocorticoid therapy is continued. Unfortunately, SSNS relapses in the majority of children when the glucocorticoids are reduced, which results in protracted, repeated courses of glucocorticoids. The standard prednisone dose for relapses is 2 mg/kg per day, which far exceeds the 5 mg/day that is considered a risk factor for GIO in adults. Although SSNS relapses are associated with transient increases in cytokines, these abnormalities promptly resolve with glucocorticoid therapy and disease remission. Therefore, we propose SSNS as a clinical model without significant systemic inflammatory involvement to examine the effects of glucocorticoids on the growing skeleton.

We examined spine and whole-body BMC in a cross-sectional study of 60 children and adolescents with established SSNS and 195 healthy controls. The subjects with SSNS had received an average of 23,000 mg of glucocorticoids over a 4-year interval, which resulted in an average dose of 0.65 mg/kg per day. Subjects with SSNS had significantly decreased height (P = .008) and increased BMI z scores (P < .001) compared with controls. The prevalence of obesity in the control group was 16%, consistent with the 15.5% prevalence of obesity in children and adolescents nationwide. In contrast, 38% of the subjects with SSNS were obese. Among the subjects with SSNS, height z score was significantly and negatively correlated with the lifetime cumulative milligrams (r = −0.28; P = .03) and cumulative milligrams per kilogram (r = −0.38; P = .003) of glucocorticoids. BMI z score was not correlated with glucocorticoid measures.

Spine BMC, adjusted for bone area, age, gender, Tanner stage, and race, did not differ significantly between patients and controls (P = .51). We had documented that obesity, in otherwise healthy children, was associated with a significant increase in whole-body, hip, and spine BMC and bone size. Therefore, these models were adjusted for BMI z score. In the adjusted model, spine BMC was 4% lower in subjects with SSNS than controls (ratio: 0.96 [95% confidence interval (CI): 0.92 to 0.99]; P = .01). Whole-body BMC, adjusted for height, age, gender, Tanner stage, and race, was 11% higher in subjects with SSNS than controls (ratio: 1.11 [95% CI: 1.05 to 1.18]; P < .001); however, the addition of BMI z score to the model eliminated the association with SSNS (ratio: 0.99 [95% CI: 0.94 to 1.03]; P = .55).

These data suggested that intermittent treatment with high-dose glucocorticoids during growth was not associated with bone deficits relative to age, bone size, gender, and maturation in SSNS. Glucocorticoid-induced obesity was associated with increased whole-body BMC and maintenance of spine BMC. Subsequent analyses were performed to examine the relations between obesity and growth in nephrotic syndrome compared with controls. Height z score was positively associated with BMI z score among subjects with nephrotic syndrome and controls. The mean height z score in those with nephrotic syndrome was −0.08 (95% CI: −0.37 to 0.21), which was significantly decreased given the degree of obesity. The overall height was normal for age because of a mitigating effect of elevated BMI on glucocorticoid-induced growth retardation.

### TABLE 3 Hierarchical Models of Whole-Body BMC z Scores in Crohn Disease

<table>
<thead>
<tr>
<th>Models</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age and race</td>
<td>−1.16 (−1.51 to −0.82)</td>
<td>−0.61 (−0.95 to −0.27)</td>
</tr>
<tr>
<td>Height, age, and race</td>
<td>−0.63 (−0.95 to −0.30)</td>
<td>−0.44 (−0.81 to −0.06)</td>
</tr>
<tr>
<td>Height, age, race, and Tanner stage</td>
<td>−0.50 (−0.85 to −0.15)</td>
<td>−0.35 (−0.72 to 0.02)</td>
</tr>
<tr>
<td>Height, age, Tanner stage, and lean mass</td>
<td>−0.19 (−0.43 to 0.06)</td>
<td>&lt;0.05 (−0.34 to 0.25)</td>
</tr>
</tbody>
</table>

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These disparate chronic childhood diseases that are treated chronically with high-dose glucocorticoids highlight the important impact of the underlying disease and its effect on growth and nutrition. These analyses also demonstrate the importance of concurrent healthy control to adjust for differences in growth and body composition across the broad age range of subjects. It is critical to note that the absence of a bone deficit after adjustment for lean mass in the subjects with Crohn disease does not imply that the bones are normal or adequate. Growth, in the absence of normal loading, results in bones that are adapted to their diminished functional requirement, with decreased mass, size, and strength. These bones may be inadequate to withstand even minor trauma.

A study is currently underway in an inception cohort of children at the time of diagnosis of Crohn disease or SSNS. In the study we are examining bone (as measured by QCT), body composition, growth, maturation, and cytokine levels before glucocorticoid therapy and during the first year of therapy. The accurate characterization of glucocorticoid and disease effects on skeletal development is necessary to identify and evaluate targeted therapies to optimize skeletal architecture and peak bone mass.

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