Pediatric Graduate School
Hospital for Children and Adolescents
University of Helsinki
Helsinki, Finland

BONE HEALTH IN CHILDREN AND ADOLESCENTS WITH JUVENILE IDIOPATHIC ARTHRITIS AND SOLID ORGAN TRANSPLANT

Helena Valta

ACADEMIC DISSERTATION

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SUPERVISORS

Docent Outi Mäkitie
Department of Pediatric Endocrinology
and Metabolic Bone Diseases
Hospital for Children and Adolescents
University of Helsinki
Helsinki, Finland

Professor Hannu Jalanko
Department of Pediatric Nephrology
and Transplantation
Hospital for Children and Adolescents
University of Helsinki
Helsinki, Finland

REVIEWERS

Docent Marja Ala-Houhala
Department of Pediatrics
Tampere University Hospital
Tampere, Finland

Professor Matti J Välimäki
Division of Endocrinology
Department of Medicine
Helsinki University Central Hospital
Helsinki, Finland

OPPONENT

Professor Ilkka Pörsti
Medical School
Internal Medicine
University of Tampere
and
Department of Internal Medicine
Tampere University Hospital
Tampere, Finland

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To my family

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ABSTRACT

Osteoporosis is a skeletal disorder characterized by compromised bone strength that predisposes to increased risk for fractures. Peak bone mass is an important determinant of adult bone health and lifetime risk for fractures. Peak bone mass is mostly acquired by the age of 18 years, and therefore, childhood and adolescence are critical periods for bone mass gain. Long-lasting childhood disease may reduce the rate and/or magnitude of skeletal mass accrual; medications, especially glucocorticoids (GCs), chronic inflammation, decreased physical activity, hormonal deficiencies, delayed puberty, and poor nutrition may predispose these children and adolescents to impaired bone health. The improved long-term outcome of severe childhood diseases has increased the number of young patients at risk of skeletal complications.

In this work, we studied overall bone health and the incidence and prevalence of fractures in children and adolescents treated for juvenile idiopathic arthritis (JIA) or who had undergone solid organ transplantation (Tx). The clinical and treatment-related factors that predispose these patients to impaired bone health were assessed. The role of especially GCs and vitamin D in this process was evaluated.

The first study cohort included 62 patients diagnosed with JIA at least two years prior to the study and treated with GCs. The epidemiology of fractures after Tx was investigated in 196 patients and a more detailed analysis of bone health determinants was performed on 40 liver (LTx) and 106 renal transplantation (RTx) patients. Clinical, biochemical, and treatment-related factors were evaluated. Bone mineral density (BMD) and vertebral morphology were assessed by dual-energy X-ray absorptiometry. Standard radiographs were obtained to detect vertebral fractures and to determine bone age; BMD values were adjusted for skeletal maturity.

Median BMD values were subnormal in all patient cohorts. The values were highest in patients with JIA (median for lumbar spine BMD Z-score -0.4) and lowest in patients with LTx (median Z-score -1.2). Age at transplantation influenced BMD values in LTx but not RTx patients; BMD values were higher in patients who had LTx before the age of two years, while no difference was found in patients who had RTx before the age of two years and in those diagnosed at an older age. Further, BMD was lowest immediately after Tx, but tended to improve during follow-up despite long-term immunosuppressive medication. BMD Z-score decreased by $\geq$ 0.7 SD in 80% of LTx patients during puberty, and was significantly subnormal (mean LS Z-score -1.9) in all LTx patients at completion of puberty. However, the median BMD Z-scores increased in male RTx subjects, while in females the trend was to decrease. The prevalence of vertebral fractures ranged from 10% to 19% in the cohorts. Most of the fractures were

asymptomatic and diagnosed only at screening. The prevalence of vertebral fractures was highest in adolescent LTx patients, and patients with fractures were older at the time of Tx ($p = 0.002$) and had more recently received the transplant ($p = 0.019$).

Vitamin D deficiency was common in all patient groups, and despite vitamin D substitution, only one-fourth of RTx and LTx patients achieved the target serum 25-hydroxyvitamin D concentration of 80 nmol/L. Several potential risk factors for compromised bone health were identified in the cohorts, such as age of the patient at onset of JIA or Tx, time since Tx or JIA diagnosis, immunosuppressive drugs, and vitamin D and parathyroid hormone concentrations. However, none of these factors alone could be regarded as the main determinant of BMD or fractures. The total cumulative weight-adjusted dose of GC was not associated with BMD values in JIA or LTx patients. The combination of female gender and age over 15 years, parathyroid hormone concentration over 100 ng/L, and cumulative weight-adjusted methylprednisolone dose over 150 mg/kg during the three preceding years were found to be important predictors for low lumbar spine BMD in RTx patients.

Our study showed that impaired bone health, manifested as low BMD and increased fracture rate, is an important health concern in children with JIA, especially after solid organ transplantation. Special attention should be directed to preventive measures during the immediate posttransplantation years and during adolescence since the skeleton seems to be most vulnerable during these times. Vertebral fractures were common but often asymptomatic. As vertebral fractures are a sign of compromised bone quality, they should be actively screened and detected early. More attention should be paid to adequate calcium and vitamin D substitution in patients with JIA and after Tx. Careful follow-up is required to ensure optimal bone mass gain also in these children and adolescents. Further studies are warranted to evaluate optimal preventive measures and treatment modalities in pediatric patients with impaired bone health.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by Roman numerals I-IV:

I Valta H, Lahdenne P, Jalanko H, Aalto K, Mäkitie O. Bone health and growth in glucocorticoid-treated patients with juvenile idiopathic arthritis.
J Reumatol. 2007; 34:831-836.

II Helenius I, Remes V, Salminen S, Valta H, Mäkitie O, Holmberg C, Palmu P, Tervahartiala P, Sarna S, Helenius M, Peltonen J, Jalanko H. Incidence and predictors of fractures in children after solid organ transplantation: a 5-year prospective, population-based study.
J Bone Miner Res. 2006; 21:380-387.

III Valta H, Jalanko H, Holmberg C, Helenius I, Mäkitie O. Impaired bone health in adolescents after liver transplantation.
Am J Transplant. 2008; 8:150-157.

IV Valta H, Mäkitie O, Rönnholm K, Jalanko H. Bone health in children and adolescents after renal transplantation.
J Bone Miner Res. 2009, May 6 (Epub ahead of print).

 Some previously unpublished data are also presented.

ABBREVIATIONS

$1,25(OH)_2D$	1,25-dihydroxyvitamin D, calcitriol
$24,25(OH)_2D$	24,25-dihydroxyvitamin D
25-OH-D	25-hydroxyvitamin D, calcidiol
aBMD	Areal bone mineral density
Aza	Azathioprine
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CHAQ	Childhood Health Assessment Questionnaire
CKD	Chronic kidney disease
CsA	Cyclosporine A
D_2	Ergocalciferol
D_3	Cholecalciferol
DXA	Dual-energy X-ray absorptiometry
FK506	Tacrolimus
GC	Glucocorticoid
GFR	Glomerular filtration rate
GH	Growth hormone
HTx	Heart transplantation
IGF-1	Insulin-like growth factor 1
ILAR	International League of Associations for Rheumatology
IVA	Instant vertebral assessment
JIA	Juvenile idiopathic arthritis
LRP5	Low-density lipoprotein receptor-related protein
LS	Lumbar spine
LTM	Lean tissue mass
LTx	Liver transplantation
MMF	Mycophenolate mofetil
MP	Methylprednisolone
MTX	Methotrexate
NIH	National Institutes of Health
OI	Osteogenesis imperfecta
OPG	Osteoprotegerin
PBM	Peak bone mass
PTH	Parathyroid hormone
RANK	Receptor activator of nuclear factor kappa B
RANKL	Receptor activator of nuclear factor kappa B ligand
RTx	Renal transplantation
SD	Standard deviation
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor alpha
Tx	Transplantation
VFA	Vertebral fracture assessment
VFx	Vertebral fracture
WHO	The World Health Organization

1. INTRODUCTION

The basis of life-long bone health is formed in childhood and adolescence. In healthy children, bone mass increases during the two first decades of life, and a rapid increase in bone mass gain occurs during puberty (Bonjour et al. 1991, Kröger et al. 1993). An estimated 90% of peak bone mass (PBM) is acquired by the age of 18 years, and PBM is an important determinant of adult bone health (Bailey et al. 1999, Heaney et al. 2000). Chronic disease in childhood may interfere with bone mass accrual, and genetically predetermined PBM might not be achieved. Inflammation, hormonal disturbances, delayed pubertal maturation, medications, especially glucocorticoids (GCs), impaired physical activity, and nutritional problems may predispose a child or adolescent to impaired bone health (Sochett and Mäkitie 2005, Bianchi 2007).

Juvenile idiopathic arthritis (JIA) is the most commonly diagnosed rheumatic disease in children. The annual incidence of JIA in Finland is approximately 21 per 100 000 children, and the prevalence in Scandinavia has been estimated to be 1 per 1000 children (Gare and Fasth 1992, Moe and Rygg 1998, Berntson et al. 2003). Solid organ transplantation (Tx) has become a successful treatment modality for children and adolescents with end-stage renal disease, terminal liver failure, metabolic disease, malignancy, and treatment-resistant heart failure. Outcomes of Tx have clearly improved during the last 20 years due to better surgical techniques, advanced intensive care treatment, and developments in immunosuppressive therapy. The number of children and adolescents living with a graft is increasing. By February 2009, a total of 204 kidney, 96 liver, and 59 heart transplantations had been performed in Finland on patients aged under 18 years.

Low bone mineral density (BMD) and fragility fractures are serious long-term complications associated with JIA (Varonos et al. 1987, Lien et al. 2003, Burnham et al. 2006a). The underlying renal or liver disease prior to Tx may itself have negative effects on the skeleton such that osteoporosis and fractures may be detected already at the time of Tx. Postoperatively, a further decline in BMD values has been reported (Feber et al. 1994, Guthery et al. 2003, Hardinger et al. 2003, Gasser 2008, Rodriguez-Garcia et al. 2009).

Preliminary data in children and adolescents with JIA or a history of Tx suggest that these patients may be prone to impaired bone health and fractures. However, the prevalence and characteristics of impaired bone health as well as the contributing risk factors remain largely unknown. We therefore carried out this study to evaluate bone health characteristics and to determine specific risk factors for skeletal problems in patients with GC-treated JIA or solid organ Tx during childhood or adolescence.

2. REVIEW OF THE LITERATURE

2.1 Bone structure

The skeleton, composed of bone and cartilage, gives mechanical support to the body and together with muscles, tendons, and joints facilitates locomotion. It also protects vital internal organs and bone marrow from potentially harmful exogenous effects. The skeleton serves as a storage site for calcium, phosphate, and magnesium, and a buffering site for excess hydrogen ions (Demster 2006).

Macroscopically, bone can be divided into two subtypes. Cortical or compact bone constitutes about 80% of the total skeleton and is mainly found in the diaphyseal parts of long bones. About 20% of the skeleton is trabecular bone, also called cancellous or spongy bone. This bone type is predominantly present in flat bones, vertebrae, and the metaphyseal and epiphyseal segments of long bones. The ratio of cortical and trabecular bone is 25:75 in the vertebrae and 95:5 in the radial diaphysis (Clarke 2008). These bone types differ in metabolic activity. Trabecular bone is metabolically more active so that 25% of it is replaced annually, in contrast to only 2-5% of cortical bone. Cortical bone is very compact and mostly calcified, and its main function is mechanical and protective (Demster 2006).

Bone is composed of mineral (50-70%), organic components (20-40%), water (5-10%), and lipids (<3%). Hydroxyapatite comprises 95% of the mineral component, and the remainder consists mostly of calcium carbonate, magnesium, and citrate. The organic matrix is mainly composed of type 1 collagen (90%), with the remaining 10% consisting of noncollagenous proteins such as glycoproteins, osteocalcin, osteonectin, sialoprotein, osteopontin, and various proteoglycans. Only 2% of the organic component of bone is composed of bone cells responsible for bone formation, resorption, and maintenance of the remodeling process (Demster 2006, Clarke 2008).

2.2. Bone cells

2.2.1 Osteoblasts

Osteoblasts are derived from bone marrow stromal cells or connective tissue mesenchymal cells that differentiate first into preosteoblasts and further into mature osteoblasts (Figure 1A). They are responsible for bone formation and synthesize and secrete collagen and matrix proteins. Cytokines and many hormones regulate their activity. The osteoblasts synthesize osteoid, the organic bone matrix, and later take care of its mineralization. Their cytoplasms contain large amounts of alkaline phosphatase. Osteoblast lineage cells secrete osteoprotegerin (OPG), an inhibitor of osteoclastogenesis, and have receptors for receptor activator of nuclear factor kappa B ligand (RANKL), 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], and parathyroid hormone (PTH), all of which can promote osteoclast activity and bone turnover. At the end of the secreting period, osteoblasts become calcified and lay in bone lacunae. Approximately 10-15% of osteoblasts are transformed later to osteocytes. Osteoblasts have traditionally been thought to be the main target of PTH action in the skeleton (Harada and Rodan 2003, Aubin 2006).

2.2.2 Osteocytes

Osteocytes are transformed from osteoblasts and account for over 90-95% of all bone cells in the skeleton (Figure 1A). They are thought to respond to mechanical strain and transmit signals of bone formation and resorption (Bonewald and Johnson 2008). Osteocytes are found throughout the mineralized matrix, especially in cortical bone, but also in the trabecular bone. They are connected to each other and to surface osteoblasts via dendritic processes (Kamioka et al. 2001). Unlike osteoblasts and osteoclasts, both of which have one specific function, osteocytes appear to be multifunctional. Remodeling is also suggested to be controlled by signals mediated by osteocytes, and osteocytes play an important role in the repair of bone microdamages. Empty lacunae are observed in aging bone, and osteocytes are proposed to undergo apoptosis; bone loss may be partly due to osteocyte cell death (Bonewald 2004).

2.2.3 Osteoclasts

Osteoclasts are derived from hematopoietic monocyte/macrophage precursor cells in the bone marrow and are responsible for bone resorption (Figure 1B). Cytokines, RANKL, and macrophage colony-stimulating factor are essential for basal osteoclastogenesis.

Monocytes circulate in blood and are attracted to the bone surface where they fuse to form osteoclasts. Osteoclasts secrete large quantities of matrix metalloproteinases and cathepsins, which are crucial for the removal of the bone's organic matrix. Degradation products, such as collagen fragments, inorganic phosphate, and calcium, are processed within the osteoclasts and then released into the circulation (Teitelbaum 2000, Boyle et al. 2003).

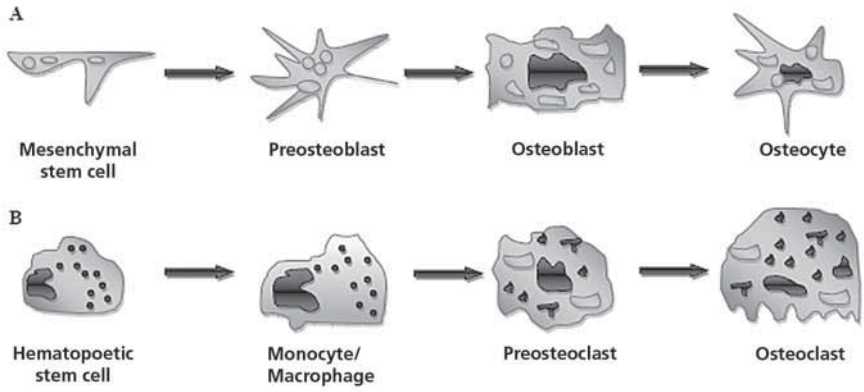

Figure 1. Origin of osteoblasts, osteocytes (A), and osteoclasts (B).

2.3 Bone modeling and remodeling

The shape and structure of bones are continuously modified and revised by two separate processes: modeling and remodeling.

2.3.1 Modeling

Modeling refers to a process in which bones change their overall shape by the independent actions of osteoblasts and osteoclasts in response to physiological stimuli or mechanical forces. This process maintains the normal shape of the tubular bones and results in an increase of the bone circumference during growth in children and adolescents. However, it occurs also in adults in response to mechanical loads, to change the shape of the bone. In the modeling process, bone formation is independent of preceding bone resorption; osteoblasts are continuously active during the process, and therefore, rapid increases in the amount of bone tissue are possible. Osteoclasts remove less bone than is formed by osteoblasts, and this leads to a net increase in the amount of bone tissue (Demster 2006, Boyce and Xing 2008, Clarke 2008).

2.3.2 Remodeling

Bone remodeling is a lifelong process consisting of successive cycles of bone resorption and formation that renew the bone while maintaining its structure (Figure 2). The regulation involves an interplay between systemic hormones, mechanical stimuli, and locally produced cytokines, growth factors, and other mediators (Compston 2001). Remodeling takes place in the basic bone multicellular units along the trabecular surface and within the cortical bone. During a remodeling cycle osteoclasts remove a small quantity of bone, which is, after a reversal time, replaced by new tissue formed by osteoblasts (Boyce and Xing 2008). There is usually a balance between the removed and newly formed amounts of bone. In healthy young adults, the remodeling balance is close to zero such that the total amount of bone tissue remains largely unchanged. Bone loss occurs when the overall remodeling balance remains negative, and the amount of bone formed is less than the amount removed by bone resorption. The rate of bone remodeling and the number of bone multicellular units are increased in for example postmenopausal osteoporosis, hyperparathyroidism, and rheumatoid arthritis. The remodeling process is vital for the repair of microdamage and maintenance of skeletal integrity, but it does not result in changes in bone shape (Boyce and Xing 2008, Clarke 2008).

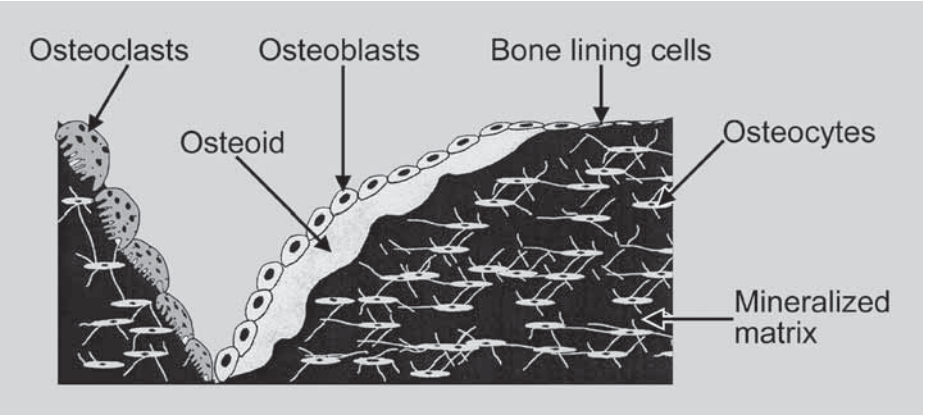

Figure 2. Bone remodeling cycle.
During the first phase, osteoclasts break down old bone matrix and a resorption cavity is formed. During the second phase, osteoblasts fill the cavity with collagen and other matrix proteins. During the third phase, the collagenous matrix undergoes mineralization, and during the resting phase lining cells cover the bone surface.

2.3.3 OPG/RANKL/RANK system

RANKL is an essential cytokine for osteoclastogenesis. It is a member of the tumor necrosis factor (TNF) family and expressed by osteoblasts and their immature precursors (Figure 3). Receptor activator of nuclear factor kappa B (RANK) is a receptor of RANKL and expressed by osteoclast lineage cells. It promotes osteoclast formation and activation, and suppresses osteoclast apoptosis, thereby prolonging osteoclast survival time (Hofbauer and Schoppet 2004). OPG acts as a soluble decoy receptor for RANKL and is able to block its effects. Various tissues and cell types, including osteoblasts, produce OPG; it protects bone from excessive resorption by binding to RANKL. OPG-deficient mice demonstrate decreased BMD, whereas the overexpression of OPG in transgenic mice leads to osteopetrosis (Bucay et al. 1998). Alterations in the OPG/RANKL/RANK system are related to metabolic bone diseases in humans. Specific RANKL inhibitors, including a monoclonal antibody to RANKL, have been developed during the last few years. They have also been tested in humans in clinical trials showing successful inhibition of bone resorption, but the long-term effects are unknown and further studies are needed (Boyce and Xing 2008).

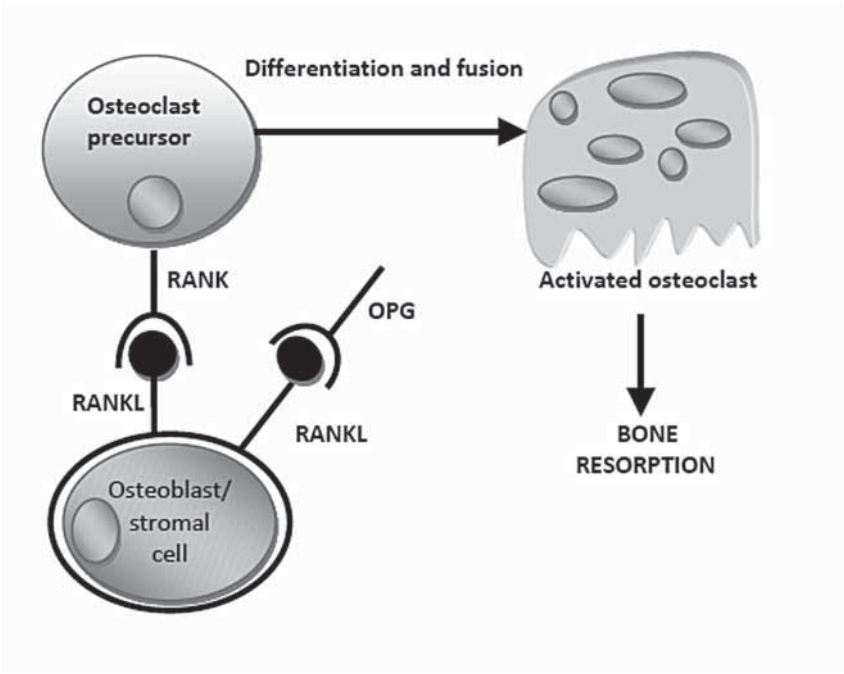

Figure 3. OPG-RANK-RANKL system.

2.4. Bone mass accrual in childhood and adolescence

Skeletal mass increases from approximately 70-95 g at birth to 2400 and 3300 g in young women and men, respectively (Trotter and Hixon 1974). Childhood and adolescence are characterized by longitudinal growth, resulting from a combination of bone modeling and remodeling, as well as changes in skeletal shape and size.

Mechanical forces on bone, generated by muscles, tightly control bone mass accrual (Frost and Schonau 2000). PBM is the amount of bone tissue accrued at the end of skeletal maturation (Bonjour et al. 1994). An estimated 70-80% of the variance in PBM between individuals is determined by genetic factors and the rest by environmental factors. These estimates are based on comparisons of BMD between monozygotic and dizygotic twins, and between twins and nontwin siblings (Dequeker et al. 1987, Pocock et al. 1987, Young et al. 1995, Eisman 1999, Albagha and Ralston 2003).

In healthy children, bone mass increases throughout the first two decades of life. However, the most rapid rise occurs during puberty in both genders, at 11-13 years in girls and at 13-17 years in boys (Bonjour et al. 1991, Kröger et al. 1993). In girls, the rate of bone mass gain decreases rapidly after menarche, while in boys bone mass gain persists up to the age of 17 years (Theintz et al. 1992). The timing of puberty may also be important for the magnitude of PBM. Late menarche in girls and delayed puberty in boys have in some studies been associated with reduced BMD (Rosenthal et al. 1989, Finkelstein et al. 1992, Bertelloni et al. 1995).

Before puberty, boys and girls develop bone mass at similar rates, but during puberty and early adulthood boys tend to acquire more bone mass (Nelson et al. 1997, Nguyen et al. 2001). Sex steroids are the key regulators of skeletal growth and maturation during puberty. In girls, estrogen initiates together with growth hormone (GH) and insulin-like growth factor 1 (IGF-1) the 3- to 4-year pubertal growth spurt, during which the bone mass almost doubles. Estrogen is also required for the attainment of maximal PBM and is essential for normal closure of the growth plates in both genders (Compston 2001). The action of testosterone induces larger bone size and approximately 25% greater areal BMD (aBMD) in boys than in girls (Riggs et al. 2002).

The PBM is known to be an important determinant of the future bone health and fracture risk; at least 90% of PBM is acquired by the age of 18 years (Bailey et al. 1999, Heaney et al. 2000). The full genetic potential of PBM is achieved only if physical activity, calcium and vitamin D intake, and nutrition are optimized and growth and pubertal development occur normally. The impact of nonoptimal lifestyle factors may be most harmful during adolescence, the pe-

riod during which the bone mass mostly accrues. A chronic illness may have deleterious effects on bone health by various mechanisms, including underlying illness, medications, associated secondary hypogonadism, and delayed pubertal maturation (Sochett and Mäkitie 2005, Bianchi 2007). These effects may be immediate, resulting in fragility fractures during childhood, or become evident with a delay after suboptimal attainment of PBM and subsequent susceptibility to adult osteoporosis.

2.5. Regulators of bone mass

2.5.1 Calcium

The genetic potential of bone mass cannot be reached or maintained if calcium supply from the diet or its absorption from the intestine is insufficient. During childhood and adolescence calcium is also needed for skeletal growth. Inadequate calcium intake results in low calcium absorption, lower circulating ionized calcium levels, and increased PTH secretion (Dawson-Hughes and Bischoff-Ferrari 2007). PTH regulates blood calcium levels by releasing calcium from bone and stimulating its reabsorption in the kidney, as well as by stimulating the renal synthesis of $1,25(OH)_2D$, with a consequent increase in intestinal calcium absorption. In addition to depleting or limiting bone mass gain, low calcium intake causes bone fragility through the PTH-stimulated increase in bone remodeling (Heaney et al. 2000). Calcium is a threshold nutrient so that below a certain level skeletal accumulation varies with intake, and above this level the accumulation is constant regardless of increased intake (Heaney 2007).

The dietary recommendations of the National Nutrition Council (2005) in Finland for daily calcium intake in children and adolescents are presented in Table 1.These recommended doses are below the threshold of calcium intakes suggested by Matkovic and Heaney (1992) and Jackman et al. (1997). A positive correlation between milk consumption in childhood and adolescence and BMD in adulthood has been observed (Murphy et al. 1994, Soroko et al. 1994, Teegarden et al. 1999). However, some studies in healthy children and adolescents have found neither a correlation between total dietary calcium consumption and childhood bone health nor consistent benefits from increased intake of dairy products for children's bone health (Kröger et al. 1993, Kardinaal et al. 1999, Jones et al. 2000). Other studies, by contrast, have reported these factors to have a positive effect (Ruiz et al. 1995, Goulding et al. 1998, Wang et al. 2003). Courteix et al. (2005) showed that 800 mg calcium substitution increased the effect of physical exercise on bone mineral gain in prepubertal girls, but this effect was not seen in the group that was less active. In a placebo-control-

led study of 51 premenarcheal twin pairs randomized to receive either 1200 mg calcium substitution daily or placebo, a small increase in hip and lumbar spine BMD was found over the next 12-18 months, but these gains were not maintained at 24 months (Cameron et al. 2004).

Table 1. Recommendation for calcium and vitamin D intake in children and adolescents according to the Finnish National Nutrition Council (2005).

Age	Calcium (mg)	Vitamin D (μg)*
6-11 months	540	10
12-23 months	600	10
2-5 years	600	7.5
6-9 years	700	7.5
10-17 years	900	7.5

* 1 μg = 40 IU

2.5.2 Vitamin D

Vitamin D metabolism

Vitamin D_2 or ergocalciferol is a plant or yeast-origin vitamin D, with wild mushrooms and algae being its main sources. Vitamin D_2 is less potent and has a shorter duration of action than vitamin D_3 (Armas et al. 2004). After sunlight exposure, ultraviolet B photons in humans penetrate the epidermis and photolyze 7-dehydrocholesterol into previtamin D_3, which rapidly becomes a more thermodynamically stable molecule, vitamin D_3 (Holick 2003). D_3 or cholecalciferol is obtained from such food items as fatty fish or fortified margarines and spreads. Increased skin pigmentation, use of sunscreens, and aging reduce the production of previtamin D_3.

Vitamin D_3 is metabolized twice to be activated. First, it is hydroxylated in the liver into 25-hydroxyvitamin D (25-OH-D) or calcidiol, which is the most abundant vitamin D metabolite in humans. The second hydroxylation, 1α-hydroxylation, occurs in the kidney, resulting in the synthesis of the active hormonal form of vitamin D, 1,25$(OH)_2$D or calcitriol (Figure 4). Both calcidiol and calcitriol undergo also 24-hydroxylation to form 24,25-dihydroxyvitamin D [24,25 $(OH)_2$D] (Holick 2003, Lips 2006). 1,25$(OH)_2$D is able to enter cells, bind to intracellular vitamin D receptor, and activate numerous target genes. The main effects of 1,25 $(OH)_2$D are to increase calcium absorption in the gut, maintain serum calcium level within the normal range, and ensure normal bone mineralization. PTH stimulates the production of calcitriol, while calcium and phosphate decrease it.

Calcitriol influences not only calcium homeostasis and bone mineralization but has also antiproliferative effects, and vitamin D deficiency may play a role in the pathogenesis of autoimmune diseases (Holick 2007). Although most of the calcitriol production occurs in the proximal renal tubule, extrarenal 1α-hydroxylase expression has been demonstrated in bone, vascular smooth muscle cells, brain, testis, prostate cells, colon epithelial cells, islet cells of the pancreas, macrophages, and parathyroid glands, and conversion of circulating 25-OH-D to its active form occurs also in these tissues (Segersten et al. 2002, Somjen et al. 2005).

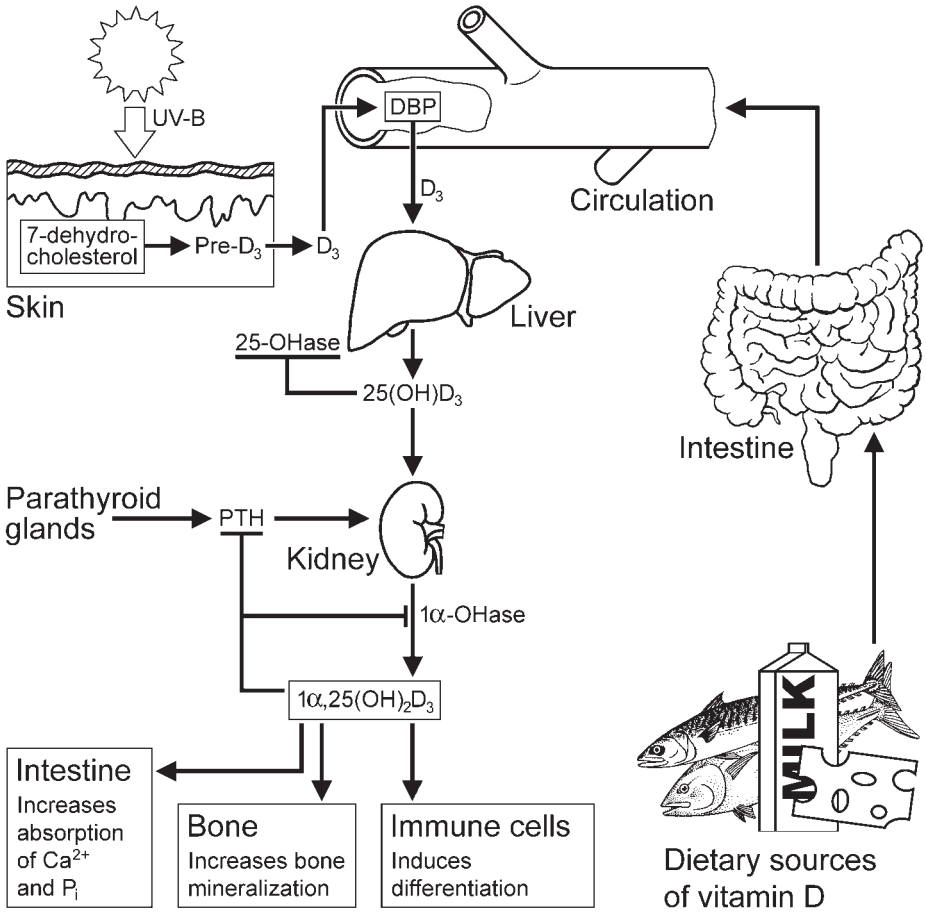

Figure 4. Metabolism of vitamin D (adapted from Deeb et al. 2007).

Vitamin D deficiency

During childhood vitamin D deficiency causes rickets, which is characterized by failure or delay in endochondral ossification at long bone growth plates (Wharton and Bishop 2003). Rickets can manifest only prior to the fusion of the epiphysis, and thus, is a disease of childhood. Clinical symptoms include swollen growth plates in long bones, knock-knees or bowed legs, enlargement of the costochondral junctions of ribs, hypotonia, and delayed motor development (Bishop 1999, Wharton and Bishop 2003). In adults, vitamin D deficiency induces osteomalacia, which is characterized by defective mineralization of osteoid on the cortical and trabecular surfaces of bone and an increased amount of unmineralized osteoid. Both rickets and osteomalacia may be associated with bone pain, muscle weakness, and hypocalcemic seizures. The typical laboratory findings in vitamin D deficiency rickets/osteomalacia are hypocalcemia, normal or low phosphate, elevated PTH, and increased alkaline phosphatase

Serum 25-OH-D concentration reflects vitamin D status in the body. The half-life of 25-OH-D is much longer than that of 1,25 $(OH)_2D$, 14-21 days versus 4-6 hours (Lauridsen et al. 2005). The target serum levels for optimal vitamin D status have increased during the last years. The S-25-OH-D threshold for maximal calcium absorption capacity is suggested to be 80 nmol/L, and this level has been regarded as the ideal minimum vitamin D concentration for optimal bone health (Heaney et al. 2003, Dawson-Hughes et al. 2005). The concentration of 80 nmol/L has been shown to prevent bone loss, decrease fracture risk, and suppress PTH secretion in the elderly (Dawson-Hughes et al. 2005).

The daily recommendations for vitamin D intake in Finland, according to the National Nutrition Council (2005) are presented in Table 1. Healthy children and adolescents in Finland have been demonstrated to have poor vitamin D status, especially during wintertime. The vitamin D level was ≤ 37.5 nmol/L in 75% of 171 healthy Finnish girls, aged 9-15 years during winter (Lehtonen-Veromaa et al. 2002). Välimäki et al. (2004) found that 39% of 220 healthy Finnish military recruits had vitamin D values below 20 nmol/L in winter. Viljakainen et al. (2006) showed in a double-blind placebo-controlled study comprising 228 girls, aged 11-12 years, that vitamin D substitution of 5 µg and 10 µg increased bone mass accrual in the hip by 14.3% and 17.2%, respectively, compared with the placebo group. In the lumbar spine (LS), only the higher 10-µg dose increased bone mineral content significantly. It has been suggested that the recommendations for daily vitamin D intake in Finland be re-evaluated since insufficient vitamin D status negatively impacts PBM accrual (Välimäki et al. 2004) and may even have negative effects on the extraskeleton.

2.5.3 Physical activity

Bone mass accrual is tightly controlled by mechanical loads on bone generated by muscle forces (Frost and Schonau 2000). The beneficial effects of regular exercise on bone mass accrual during childhood and adolescence have been confirmed in many studies (Bailey et al. 1999, Nurmi-Lawton et al. 2004, Hasselstrom et al. 2007). The maximal positive effect on BMD may require quite intense loading with high impact forces. Weight-bearing exercise, such as walking, dancing, running, jogging, jumping, and aerobics, is considered most effective. The exact duration or intensity of physical activity that provides the optimal stimulus for bone mass accrual is unclear. In one study, gymnasts had higher spinal and femoral BMD compared with swimmers or sedentary girls (Courteix et al. 1998). Differences in BMD values between the dominant and nondominant arm in junior tennis players were found, and if the training program was started already in childhood, also the diameter of the dominant forearm was increased (Haapasalo et al. 1998). Whether the positive effects of physical activity on the growing skeleton are maintained during adulthood remains to be elucidated. Baxter-Jones et al. (2008) found that physically active adolescents, as compared with their less active peers, maintained their higher bone mineral content (BMC) into the third decade of life. In a Japanese study, 46 postmenopausal women who had participated in weight-bearing sports between 12-18 years had significantly greater BMC in the lumbar spine and femoral neck and also greater femoral diaphyseal bone cross-sectional area than their less active peers (Kato et al. 2009). Impaired physical activity is common in chronically ill children and may negatively affect their BMD.

2.5.4 Estrogen, testosterone, and growth hormone

The period prior to puberty bone growth is largely GH-dependent, but sex steroids are essential for the completion of epiphyseal maturation and bone mineral increase during puberty. Trabecular bone is influenced more than cortical bone by sex steroids (Bass et al. 1999). Patients with hypogonadism have reduced BMD and increased fracture risk, and hormonal replacement therapy is associated with BMD improvement (Saggese et al. 1997). Boys with aromatase deficiency or estrogen receptor defects have low BMD (Khosla et al. 2002), confirming the significant role of estrogen also in boys for normal bone mass accrual. Secondary hypogonadism and delayed pubertal maturation may be associated with a chronic illness and predispose to osteoporosis.

GH deficiency in children and adolescents is associated with decreased bone mass gain and low BMD (Baroncelli et al. 2000). Adequate GH treatment leads to increased bone mass (Boot et al. 1997, Baroncelli and Saggese 2005). The GH action on bone is mediated mainly through IGF-1, which positively affects the osteoblasts and stimulates the synthesis of collagen. In GH-deficient children

and adolescents, low bone mass is partly due to reduced bone size and delayed skeletal maturation.

2.5.5 Parathyroid hormone

PTH is an 84-amino-acid peptide released from the parathyroid glands in response to hypocalcemia. Its main function is to maintain a constant plasma ionized calcium concentration. A reduction in plasma calcium increases PTH secretion, while low phosphate or elevated calcium decreases it. The three physiological regulators of PTH secretion and synthesis are extracellular calcium and phosphate ions and calcitriol. In bone, PTH stimulates calcium and phosphate release, and in the kidney tubulus it stimulates reabsorption of calcium and exretion of phosphate. It also enhances renal synthesis of 1,25 $(OH)_2D$, thereby increasing calcium and phosphate absorption from the gut (Brown 2006).

The action of PTH on bone is dual: stimulation or inhibition of bone collagen and synthesis of matrix. Chronic administration or increased secretion of PTH in primary hyperparathyroidism leads to a catabolic state, which stimulates bone resorption and leads to bone loss. The release of calcium is accompanied by the release of phosphate and matrix components. Paradoxically, when PTH is administered intermittently in suitable doses, it increases the volume of trabecular bone. These anabolic effects of PTH are under intensive research to develop new protocols for osteoporosis treatment and prevention (Potts 2005). A recombinant human PTH (1-34, teriparatide), given subcutaneously, was found to reduce the number of new vertebral fractures by 65% in postmenopausal women (Neer et al. 2001). The use of teriparatide is contraindicated in children because of the potential risk of osteosarcoma.

In the kidney, PTH inhibits phosphate reabsorption in both the proximal and distal tubules, while calcium reabsoption is increased only in the distal tubulus. In patients with normal kidney function, PTH induces phosphate excretion in urine, which can lead to hypophosphatemia and stimulate bone resorption (Brown 2006).

In chronic renal failure, several factors may contribute to the development of secondary hyperparathyroidism: 1) impaired phosphate elimination in the kidneys, 2) hypocalcemia, 3) impaired 1,25 $(OH)_2D$ synthesis, and 4) decreased number of calcium-sensing and vitamin D receptors in the parathyroid glands. In secondary hyperparathyroidism, PTH excretion is increased at all plasma calcium levels (Wesseling et al. 2008). Chronic kidney disease (CKD)-mineral bone disorder occurs in patients with chronic renal failure and who have 1) alterations in calcium, phosphate, and vitamin D metabolism, 2) abnormalities in bone turnover, mineralization, and volume, or 3) extraskeletal calcification (Sanchez 2008). The term renal osteodystrophy defines alterations in bone

morphology associated with CKD. Histomorphometry allows assessment of the state of bone turnover, mineralization, and bone volume (Moe et al. 2006).

2.6 Osteoporosis

2.6.1 Definition of osteoporosis

The World Health Organization (WHO) has defined osteoporosis as a skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture (WHO 1994). A redefinition was published in 2000 by National Institutes of Health (NIH), in which osteoporosis was defined as a skeletal disorder characterized by compromised bone strength, predisposing to an increased risk of fracture (NIH 2000). In clinical practice, the diagnosis of osteoporosis in adults is often based only on the assessment of BMD by dual-energy X-ray absorptiometry (DXA), without previous fractures. BMD results are presented as T-scores: the measured BMD is compared with the average BMD of young adults at the time of PBM, and the difference is presented in standard deviation (SD) units. These T-scores are used to define osteoporosis and osteopenia. Osteoporosis is defined as a T-score less than or equal to -2.5, and osteopenia as a T-score between -1.0 and -2.5. A T-score less than or equal to -2.5 in conjunction with a history of low-impact fracture is considered severe osteoporosis (WHO 1994).

For children and adolescents who have not yet achieved their PBM, the use of T-score is not possible. Instead, the use of Z-scores is recommended. A Z-score is a comparison of the measured BMD with the mean BMD for gender and age in SDs. According to a position statement of the International Society for Clinical Densitometry (Rauch et al. 2008), the diagnosis of osteoporosis in children and adolescents should not be based on DXA results alone, but requires both a significant fracture history and a low BMC or BMD. A clinically significant fracture history is defined as a vertebral compression fracture, one lower extremity long bone fracture, or two or more upper extremity long bone fractures. Low BMC or aBMD is defined as a Z-score $\leq$ -2.0, adjusted for age, gender, and/or body size, as appropriate. A Z-score between -2.0 and -1.0 is defined as low-normal. For children and adolescents, the term "low BMD for chronological age" may be used if the subject's Z-score is below -2.0, stature is normal for age, and no fracture history is present.

2.6.2 Classification of osteoporosis

Primary osteoporosis

Osteoporosis in an otherwise healthy child is defined as primary and is divided into heritable disorders of connective tissue and idiopathic juvenile osteoporosis. Genetic defects influencing bone development, such as osteogenesis imperfecta (OI), Ehlers-Danlos syndrome, Marfan syndrome, osteoporosis-pseudoglioma syndrome, and homocystinuria, are the major causes of primary osteoporosis. In idiopathic juvenile osteoporosis, the underlying defect is unknown.

OI is the most common form of primary osteoporosis. OI is characterized by bone fragility and low bone mass. Mutations in the type I collagen genes (*COL1A1* or *COL1A2*) cause most of the seven different clinical subtypes (Rauch and Glorieux 2004, Glorieux 2008). However, new mutations in for example, *P3H1* and *CRTAP* genes have recently been found in rarer forms of OI (Cheung and Glorieux 2008). The severity of the disease varies widely; the most severe forms lead to perinatal death, while patients with the mildest form may even avoid fractures. The extraskeletal manifestations include blue sclerae, dentinogenesis imperfecta, skin hyperlaxity, hearing impairment, and hypermobility of the joints, but their absence does not exclude the diagnosis.

Idiopathic juvenile osteoporosis is a rare condition described by Dent and Fieldman (1965) over four decades ago with an estimated incidence of 1:100 000. It usually presents during pre-pubertal years (mostly between 8 and 12 years of age) with back pain, walking difficulties, and vertebral compression fractures in an otherwise healthy child or adolescent. It is also characterized radiologically by radiolucent areas in the metaphyses of long bones. The clinical course of this disease is variable and spontaneous improvement has been reported in most patients, but in others rapid progression may occur (Dent 1977). The genetic cause is in most cases unknown, but in a study by Hartikka et al. (2005) 3 of 20 patients with idiopathic juvenile osteoporosis had a heterozygous mutation in the low-density lipoprotein receptor-related protein 5 (*LRP5*) gene.

Secondary osteoporosis

Osteoporosis caused by an underlying illness and/or its treatment is defined as secondary. The list of causes of secondary osteoporosis in children is growing, due in part to improved long-term outcomes for children with chronic diseases. In addition to the chronic illness itself, several other factors, such as medications, chronic inflammation, impaired physical activity, hormonal causes, or nutritional problems, may predispose these patients to impaired bone health. The illnesses that may be associated with secondary osteoporosis and possible predisposing factors in these patient groups are shown in Table 2.

Table 2. Diseases associated with secondary osteoporosis.

Chronic illness	Mechanism
Rheumatological diseases	
Juvenile idiopathic arthritis, systemic lupus erythematosis, dermatomyositis	Inflammatory cytokines, glucocorticoids and calcineurin inhibitors, decreased physical activity
Neuromuscular disorders	
Cerebral palsy, Duchenne muscular dystrophy, others	Decreased physical activity, decreased muscle mass and strength
Gastrointestinal disorders	
Inflammatory bowel diseases, celiac disease, cystic fibrosis, biliary atresia, cholestatic liver diseases	Inflammatory cytokines, glucocorticoids, malabsorption, poor nutrition
Renal diseases	
Chronic kidney disease, nephrotic syndromes	Renal osteodystrophy, glucocorticoids, hormonal disturbances, vitamin D deficiency
Hematological diseases	
Leukemia, stem cell transplantation	Medications, radiation therapy, decreased physical activity, late endocrine effects
Endocrine disorders	
Klinefelter syndrome, Turner syndrome, Cushing's syndrome, gonadal dysgenesis, hyperthyroidism	Hypogonadism, glucocorticoid excess, increased bone turnover
Solid organ transplantation	
Kidney, liver, heart, and lung	Underlying diseases (renal or hepatic osteodystrophy), gluco-corticoids and other medications, decreased physical activity, hormonal disturbances
Eating disorders	
Anorexia nervosa, bulimia	Malnutrition, low calcium and vitamin D intake, hormonal disturbances

2.7 Juvenile idiopathic arthritis and bone health

In previous studies, JIA has been associated with osteoporosis, vertebral and peripheral fractures, and growth retardation (Elsasser et al. 1982, Varonos et al.1987, Simon et al. 2002, Wang et al. 2002, Celiker et al. 2003, Lien et al. 2003, Burnham et al. 2006a). One longitudinal study followed 63 children with JIA and healthy controls for 18 months. Nine of the patients had a vertebral fracture (VFx) at baseline and four experienced one during the follow-up (Elsasser et al. 1982). In a population-based study of 1939 patients with childhood-onset arthritis and 207 072 controls, the patients with arthritis had an increased risk of both vertebral and nonvertebral fractures, especially during adolescence and after the age of 45 years (Burnham et al. 2006a). A Norwegian study of 105 adolescent patients with JIA found aBMD Z-score below -1.0 in 25%, 34%, and 31% of patients for the LS, whole body, and femoral neck, respectively. Low aBMD values were more frequent in adolescents with early-onset JIA than in young adult patients with later-onset JIA. Of all patients, 71% had never been on GC treatment, but 39% of this group had low BMD values (Lien et al. 2003).

2.8 Bone health in patients with a history of solid organ transplantation

Several studies have evaluated bone health in adult Tx patients and reported osteoporosis and fragility fractures. A high prevalence of osteoporosis (T-score ≤ -2.0) for the LS and/or hip was found in 41% of 32 adults after heart transplantation (HTx) (Glendenning et al. 1999). Leidig-Bruckner et al. (2001) reported that nearly one-third of 105 patients had sustained a vertebral fracture (VF) by the end of the third year after HTx. Reduced BMD and VF after LTx are prevalent in adults (Ramsey-Goldman et al. 1999, Hamburg et al. 2000, Ninkovic et al. 2000, Leidig-Bruckner et al. 2001, Hardinger et al. 2003). Longitudinal BMD measurements showed the greatest reduction in the LS during the first six months postoperatively (Hardinger et al. 2003). Long-term follow-up (up to 15 years) demonstrated an improvement in BMD mainly during the second year post-LTx, with no deterioration thereafter (Hamburg et al. 2000). One study found VFx in 14% of 130 LTx patients in the first postoperative year, and the proportion increased to 21% by the end of the second year (Leidig-Bruckner et al. 2001). Most of the fractures occurred in the lower thoracic or lumbar region. The patients with a LS BMD T-score below -2.5 were at increased risk for VFx (Leidig-Bruckner et al. 2001). In adult RTx patients, previous studies have reported variable prevalence rates of osteoporosis and fractures. In a cross-sectional study in adults, the prevalence of osteoporosis was 53% (Durieux et al. 2002). One study revealed no bone loss after the second posttransplantation year, while another study found progressive improvement in postoperative BMD, approaching normal values after 10 years (Grotz et al. 1995, Carlini

et al. 2000). A nearly 5-fold increase in fracture incidence was reported in adult RTx patients and the frequency of VFx was over 20-fold higher as than that of the general population (Abbott et al. 2001, Vautour et al. 2004).

Data on BMD development, especially VFx in pediatric liver transplant recipients, are limited (Hill et al. 1995, Guthery et al. 2003). In a study by Hill et al. (1995), 19 (16%) of 117 children who underwent LTx sustained a total of 69 fractures in a six-year period. Forty-nine (71%) of the fractures in 13 children occurred before Tx, and 20 fractures (29%) in six patients occurred after Tx. Almost all of the fractures occurred in the ribs or long bones, no spinal radiographs were taken, and no VFx, were detected (Hill et al. 1995). In another study of 109 pediatric long-term LTx survivors, only 7% had reduced bone mass (LS BMD Z-score < -2.0). These patients had been treated for rejection at least once and had greater cumulative prednisone exposure during the preceding year than those without reduced bone mass. No fracture assessment was included in this study (Guthery et al. 2003).

Limited data on bone health implications of pediatric RTx are available. Most of the previous studies are cross-sectional BMD evaluations lacking information on fractures. Chesney et al. (1984) published the first paper on children's bone health outcome after RTx and reported a significant decrease of BMC in 11 of their 18 patients; the loss was significantly greater in patients receiving daily GC treatment than in those on alternate-day dosing. In a cross-sectional study of 83 children and adolescents receiving living-related RTx, the LS aBMD Z-score was between -1.0 and -2.5 in half of the patients and below -2.5 in one-fifth (el-Husseini et al. 2004). In another cross-sectional study, the LS BMD Z-score was below -2.0 in 45% of the 33 children; older age and longer time since RTx increased the risk for low BMD (Ellis et al. 2000). Feber et al. (1994) found in a longitudinal study of 14 children a significant decrease in BMD during the first six months postoperatively despite normal graft function. During the following 18 months the bone loss stabilized and BMD increased.

2.9 Dual-energy X-ray absorptiometry

The first DXA scanners were introduced in the late 1980s to detect postmenopausal osteoporosis. Nowadays, however, DXA measurements are increasingly performed also on children and adolescents with chronic disease or frequent fractures. Measurement sites generally used in clinical practice for adults are LS (from L1 or L2 to L4), femoral neck, and total hip (Kanis 2000).

DXA is a projectional technique that provides a two-dimensional measurement of BMC (g) and bone area (cm^2) of three-dimensional bone. BMD (cm^2), derived from equation BMC/bone area, is not the true volumetric density (g/cm^3) because no measure of bone depth is included. Therefore, children with smaller

bones will falsely establish lower aBMD than children with larger bones, despite identical volumetric bone densities because bone thickness is not measured. Further, DXA is unable to distinguish between trabecular and cortical bones (Leonard 2005).

According to the International Society for Clinical Densitometry Official Positions 2007, the pediatric DXA examination should ideally include scans of the LS (posterior-anterior) and the total body less the head (Gordon et al. 2008). The hip, including total hip and proximal femur, is not regarded as a reliable measurement site in growing children due to a significant variability in skeletal development and lack of reproducible regions of interest. The whole body measurement gives important data of the total bone mass and body composition and provides also data of the fat mass and lean (mainly muscle) body mass separately. A high correlation between muscle mass and bone mass in children is in line with the functional bone-muscle unit theory (Högler et al. 2003). BMC is thought to be one of the preferred methods of bone status assessment because of its reproducibility and lack of errors related to areal density measurements. However, the lack of reference values for children under nine years of age limits its use in children (Ellis et al. 2001, Kalkwarf et al. 2007). In primary or secondary hyperparathyroidism, in which cortical bone loss prevails, a forearm measurement may give more information, but scant pediatric reference data exist for the forearm. With short imaging time and low radiation dose, DXA is also easily performed on small children.

Bone age corresponds to pubertal maturation. Children with a chronic disease often have delayed pubertal development, and correction of the DXA result for bone age is important to avoid falsely low values. Also correction for height age is used to avoid the impact of short stature on the results. Height age is estimated by determining the age at which the fiftieth percentile on the height-for-age growth curve corresponds to the child's height. Correction for height age is problematic if a short but already pubertal adolescent is compared with a prepubertal child (Bachrach 2005).

Vertebral fracture assessment (VFA) or instant vertebral assessment (IVA) of the thoracic and LS, usually from T4 to L5, can also be obtained by some DXA instruments using a specific software with the patient in either the supine or lateral position (Figure 5). The radiation exposure is very low (from 0.11 μSv to 14.9 μSv depending on the device used) compared with conventional radiography and is therefore ideal, especially for children. Whether the resolution of VFA is adequate for analysis of vertebral morphology is unclear. Studies in adult patients have demonstrated that the sensitivity and specificity of IVA were comparable with those of spine radiographs in detecting grade 2 or 3 vertebral fractures, but not as good in lower grade fractures (Lewiecki and Laster 2006). In detecting morphological vertebral deformities, VFA has been shown to be accurate in adults in the lower thoracic and lumbar regions (Chapurlat et

al. 2006, Damiano et al. 2006). There is only one pediatric study comparing IVA with conventional radiographs, and it found good visibility of vertebrae in T8-L4, but compromised visibility in the upper thoracic region (T4-Th7); IVA was constantly inferior to standard radiographs. Only 9 (36%) of the 25 vertebral fractures found in plain radiographs were detected in IVA images (Mäyranpää et al. 2007).

Strong evidence exists that, in adults, the BMD value is a good predictor for future fracture risk. Marshall et al. (1996) found that the risk of fracture doubles with each 1 SD unit decrease in aBMD T-score. In children and adolescents, the relationship between BMD and fracture risk is not yet clear. Some studies have suggested that healthy children prone to developing osteoporosis and fractures later in life could be identified by measuring BMD before pubertal development occurs (Ferrari et al. 1998, Flynn et al. 2007). Skaggs et al. (2001) found in 100 healthy girls, aged 4-15 years, half of whom had sustained a forearm fracture, that the peripheral quantitative computed tomography measured cross-sectional area at the distal radius was 8% smaller in the fracture group (Skaggs et al. 2001). Goulding et al. (2000) measured the aBMD values of 100 girls, 3-15 years of age, after a forearm fracture and noted that those who sustained a new fracture during four-year follow-up had significantly lower total body aBMD at baseline.

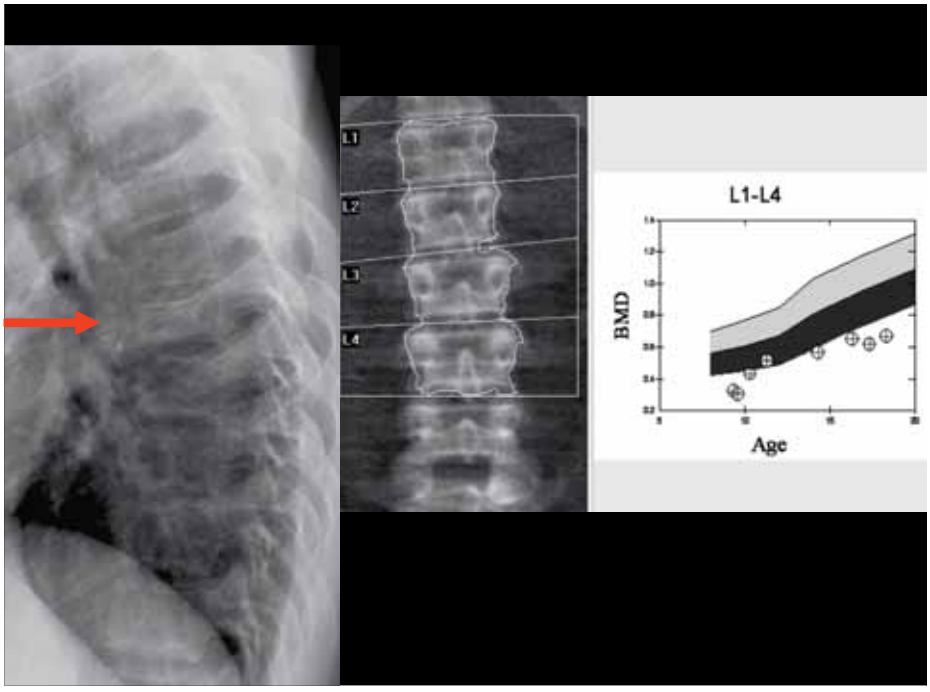

Figure 5. Vertebral fracture assessment by DXA, the red arrow on the left shows a compression fracture. Lumbar spine aBMD development with age is shown on the right.

2.10 Skeletal effects of immunosuppressive drugs

2.10.1 Glucocorticoids

Almost 80 years ago, Harvey Cushing (1932) observed that patients with excess endogenous glucocorticoid (GC) developed bone fractures. Synthetic GCs have been widely used in juvenile idiopathic arthritis (JIA) and after organ transplantations because of their anti-inflammatory and immunosuppressive potential. However, similarly to endogenous GC excess, they are known to have a strong negative influence on bone metabolism, even when administered in low doses.

GCs have both direct and indirect effects on the skeleton and cause increased bone resorption and decreased bone formation. These changes have been confirmed also histologically in bone biopsies (Dalle Carbonare et al. 2001). The skeletal effects of GCs occur in two phases. Increased bone resorption appears initially and may be due to indirect effects of GCs, including decreased intestinal calcium absorption, increased urinary calcium excretion, and decreased synthesis of gonadotrophins, sex steroids, and IGF-1. After extended exposure to GCs, the function of osteoblasts decreases and bone formation is impaired (Canalis 2003, Canalis et al. 2007). GCs increase RANKL expression, inhibit OPG production by osteoblasts, and suppress OPG serum levels (Hofbauer et al. 1999, Sasaki et al. 2001). GCs induce apoptosis of mature osteoblasts and osteocytes, resulting in a reduced number of bone-forming cells (Weinstein et al. 1998). The impact of GCs on bone is summarized in Table 3 and Figure 6.

Table 3. Mechanisms of glucocorticoid-induced bone loss.

Indirect effects	
intestinal calcium absorption	↓
urinary calcium excretion	↑
gonadotrophin synthesis	↓
sex steroid synthesis	↓
IGF-1 synthesis	↓
Direct effects	
osteoclastogenesis	↑
apoptosis of osteoblasts and osteocytes	↑
RANKL expression	↑
osteoblast life span	↓

Apoptosis of osteocytes may play an important role in GC-related BMD loss, microarchitectural deterioration of bone structure, and predisposition to fractures. Osteocytes are believed to sense the need for remodeling and communicate with lining cells (Manolagas 2000). The negative effect on bone formation leads to a reduced total amount of bone replaced in each remodeling cycle (Dalle Carbonare et al. 2005). Numerous studies in adults and animal models have demonstrated that GCs induce predominantly trabecular bone loss (Dalle Carbonare et al. 2001). GC-induced myopathy may also contribute to bone loss by altering gravitational forces on the skeleton and reducing weight-bearing activities and mobility. Secondary hyperparathyroidism does not explain GC-induced osteoporosis because in most patients PTH is normal or only slightly elevated, and GCs mainly induce trabecular bone loss whereas hyperparathyroidism is predominantly associated with cortical bone loss (Canalis et al. 2007). In children and adolescents, impaired linear growth is associated with GC therapy (Foster et al. 2004). GCs and IGF-1 have opposite effects on the skeleton; IGF-1 increases the function of osteoblasts, bone collagen synthesis, and bone formation (Giustina et al. 2008).

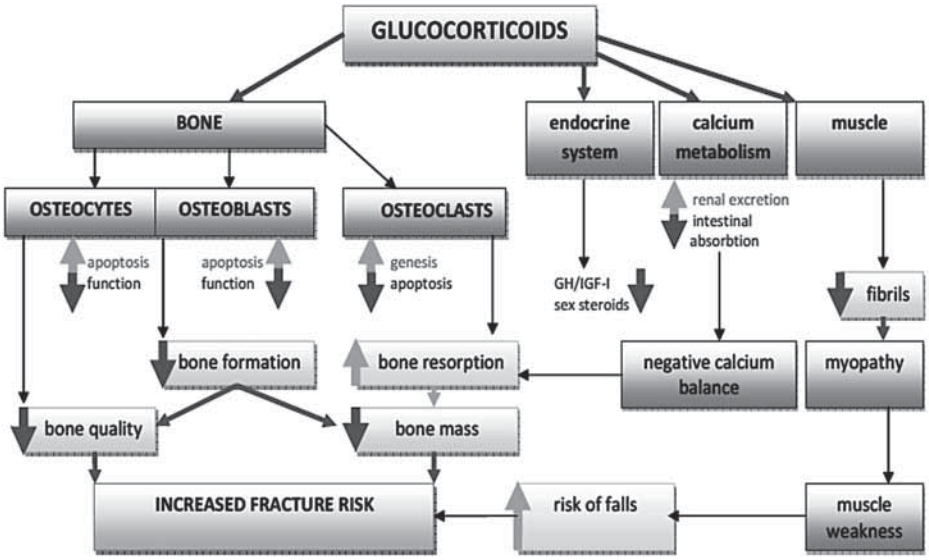

Figure 6. Glucocorticoids and bone.

GC-induced osteoporosis is the most common form of secondary osteoporosis (Mazziotti et al. 2006). The risk of fractures is increased in adult patients during the use of GCs (van Staa et al. 2000a, Kanis et al. 2004, Steinbuch et al. 2004). Decreased BMD has been described in many pediatric disorders treated with GCs, but much less is known about fractures and GC use in this population. A large population-based study reported that children who required more than four courses of oral GCs had an increased risk of fractures, especially in the

forearm, but the risk decreased and was comparable with that of nonusers after cessation of treatment (van Staa et al. 2003a). In adults, the fracture risk was increased mostly in the hip and spine (van Staa et al. 2000b).

The disease for which GC treatment is used may also contribute to the increased risk. Inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), also suppress bone formation and induce bone loss. Independent of GC therapy, increased fracture risk has been reported in adults with rheumatoid arthritis or inflammatory bowel disease (Cooper et al. 1995, van Staa et al. 2003b). The largest adult study, including over 200 000 GC users and their age- and sex-matched controls, reported that the risk of fractures was related primarily to daily rather than cumulative GC dose, so that daily doses > 2.5 mg prednisone equivalents were associated with an increased risk for both vertebral and hip fractures (Van Staa et al. 2000a) Osteonecrosis, also known as avascular necrosis, is a serious complication of GC use. It occurs most often in the proximal femur, the head of the humerus, and the distal femur. The etiology remains unknown, but may be due to fat emboli, oxidation injury, or enhanced osteocyte apoptosis (Mankin 1992, Weinstein et al. 2000, Ichiseki et al. 2005).

An alternate-day steroid regimen may induce less GC side-effects than the daily regimen (Jabs et al. 1996, Hiraoka et al. 2003, Vidhun and Sarwal 2005). In addition to dosing, other factors, such as individual variability in the response to oral GC therapy, are important and partly explain why some patients seem to be more sensitive to GC-induced obesity, metabolic changes, osteoporosis, and fractures. Genetic polymorphisms in the GC and other steroid hormone receptor genes are associated with differences in BMD development and alterations in body composition after GC exposure (Gennari et al. 2002, van Rossum and Lamberts 2004). It has also been suggested that individual variability in expression of GC-metabolizing enzymes in osteoblasts may result in higher GC exposure within bone and development of localized osteoporosis (Cooper 2004).

2.10.2 Other immunosuppressive drugs

Cyclosporine A (CsA) and tacrolimus (FK506) are calcineurin inhibitors that suppress the activation and production of T-cells and the release of interleukin-2 and other cytokines (Matsuda et al. 2000). In murine models, CsA administration causes high-turnover osteoporosis, mainly in trabecular bone, and increases osteocalcin and 1,25 $(OH)_2D$ levels. In a high-turnover state, both bone resorption and formation are increased, but resorption rate exceeds bone formation. In a rodent model, the actions of FK506 were similar to those of CsA (Movsowitz et al. 1989, Epstein 1996). The effects of calcineurin inhibitors on the human skeleton are difficult to study because these inhibitors are rarely used in isolation and usually treated patients have an underlying disease that may itself affect bone health. Comparison of CsA monotherapy with a non-CsA regimen in renal transplant patients showed that both regimens decreased BMD at the distal radius and less in the LS but no statistical difference in the amount of bone loss was found between the groups (Cueto-Manzano et al. 2003). Kidney transplant patients receiving CsA had more bone loss than patients on FK506 (Josephson et al. 2004). FK506-based regimens may improve bone health by allowing the use of lower GC doses. Chronic nephrotoxicity leading to secondary renal insufficiency is occasionally a problem in patients treated with calcineurin inhibitors and may predispose to renal osteodystrophy (Campbell et al. 2006).

Mycophenolate mofetil (MMF), azathioprine (Aza), or rapamycin do not appear to have significant skeletal side-effects (Bryer et al. 1995, Romero et al. 1995, Dissanayake et al. 1998). Methotrexate (MTX) has been reported to cause osteopenia when used to treat childhood malignancies (Mandel et al. 2004). The dosing in JIA is substantially lower and does not seem to have adverse effects on bone (Cranney et al. 2001, Cimaz 2002). TNF-α is known to be an important mediator in various inflammatory conditions and in postmenopausal osteoporosis (Feng 2005). The new anti-TNF agents, introduced at the end of 1980s for the treatment of rheumatic diseases in adults and approximately ten years later also in children, may prevent bone loss by regulating osteoclast formation and activation or by decreasing the amount of circulating cytokines (Saidenberg-Kermanac'h et al. 2004, Feng 2005).

3. AIMS OF THE STUDY

Children with JIA or a history of solid organ Tx have several potential risk factors for osteoporosis. However, the prevalence of osteoporosis and fragility fractures, and the role of specific clinical and treatment-related risk factors in the development of osteoporosis remain largely unknown. We therefore carried out a study in children with JIA and in children with an organ transplant. Specific aims were as follows:

1. To investigate the incidence and prevalence of fractures in children and adolescents with JIA or a solid organ transplant

2. To assess BMD by DXA

3. To determine the role of GC exposure and vitamin D deficiency in the development of impaired bone health

4. To identify other clinical and treatment-related factors that may predispose to poor bone health

4. MATERIALS AND METHODS

4.1 Patients and controls

The studies of this thesis comprised 62 patients with GC-treated JIA (Study I), 196 patients with a history of solid organ transplantation during childhood or adolescence (Study II), 40 pediatric LTx patients (Study III), and 106 pediatric RTx recipients (Study IV). Twenty-five and 68 of the patients in Studies III and IV, respectively, were also included in Study II.

In epidemiological Study II, fractures in children, adolescents, and young adults (from 0 to 30 years) that were treated in primary health centers and university hospitals in the city of Helsinki, were used as controls. This group comprised of 207103 individuals.

Validity of the BMD reference values, was tested in 199 healthy Finnish children ranging in age from 7 to 19 years.

4.1.1 Inclusion criteria for patients

Study I: Consecutive patients between the ages of 4 and 18 years who fulfilled the revised criteria of JIA according to the International League of Associations for Rheumatology (ILAR) classification. The time since diagnosis of JIA had to be at least 2 years, and the patient should have been on systemic GC treatment for at least 3 months and treated at the Hospital for Children and Adolescents, University of Helsinki, since diagnosis.

Study II: Children, adolescents, and young adults who had undergone RTx, LTx, or HTx under the age of 18 years during 1983-2002 and who were treated at the Hospital for Children and Adolescents, University of Helsinki. Young adults who had already been transferred to adult units for follow-up were also invited to participate.

Studies III and IV: Consecutive children and adolescents who had undergone LTx before the age of 16 years (III) or RTx before the age of 18 years (IV) at least 6 months before the date of enrollment and who were aged between 4 and 20 years at the time of the study. All transplantations had been performed at the Hospital for Children and Adolescents, University of Helsinki, and follow-up of the patients was at the same hospital. Patients on dialysis treatment were excluded (IV).

4.1.2 Treatment of patients with juvenile idiopathic arthritis

The pharmacological treatment of JIA was individualized and based on the subtype of JIA and the severity of the disease. Treatment of patients with mild disease, such as oligoarthritis without uveitis, was usually initiated using intra-articular GCs or nonsteroidal anti-inflammatory drugs or a combination of these. The most commonly used nonsteroidal anti-inflammatory in our patients was naproxen at a daily dose of 15-20 mg/kg. If the symptoms of oligoarthritis persisted or worsened, or if the patient already had a polyarticular or systemic disease, a more aggressive therapy was initiated. Oral GC, in our patients usually prednisolone, was usually started with a dose of 1-2 mg/kg/daily for some weeks, and the dose was tapered as soon as possible. An alternate-day GC regimen was preferred. Patients with a systemic disease were on high GC doses longer and also intravenous GC therapy was used in these patients. Disease-modifying anti-rheumatic drugs, such as MTX, sulfasalazine, Aza, and CsA were used as second-line drugs, with MTX most often being administered at a weekly dose from 10 to 20 mg/m^2. Twenty (33%) of our patients did not respond adequately to conventional drug treatment, and TNF-α antagonists were used for them.

4.1.3 Immunosuppressive medication after transplantation

The transplant patients were treated primarily with triple-drug immunosuppressive medication, including CsA, Aza, and methylprednisolone (MP). Since year 1999, the liver and renal transplant patients also received basiliximab as an induction. Antithymocyte globulin was used as an induction therapy in HTx. CsA was started perioperatively and the target trough levels were 300-400 µg/L after LTx or HTx, and 250-300 µg/L after RTx during the first weeks. The doses were then slowly reduced to attain levels of 70-100 µg/L at one year and thereafter. MP was given 1-3 mg/kg/day during the first postoperative days and tapered to 0.25 mg/kg/day at 2 weeks. A low-dose alternate-day MP regimen was commenced at 3-6 months in RTx patients (mean daily dose 0.12 mg/kg) and at 6 months in LTx or HTx recipients (mean daily dose 0.19 mg/kg). After the first postoperative year, the MP dose was not increased with growth. Aza was given 2 mg/kg/day for the first 2 weeks and then reduced to 1 mg/kg/day, and increased to 1.3-1.4 mg/kg/day at 3 or 6 months, when MP was changed to alternate-day dosing. Acute rejections were primarily treated with MP (3 mg/kg/day) for 5 days. If features of chronic rejection or recurrent acute rejections were observed in the renal, liver, or heart biopsies, the patients were switched to FK506 and/or MMF (II-IV).

4.2 Methods

4.2.1 Study design and data collection

All studies consisted of a retrospective part in which hospital charts were reviewed for the underlying disease and treatment characteristics, growth, pubertal development, and previous fracture history. The mean weight-adjusted (mg/kg) daily doses of MP, CsA, and Aza were calculated during the annual follow-up visits (II). Orally administered GCs were converted to prednisolone equivalents in JIA patients and all doses used after the diagnosis were recorded (I). Absolute and weight-adjusted cumulative doses of MP, including the doses used for rejection treatment, were calculated for each transplant patient. For LTx patients, the whole postoperative period was included and for RTx recipients a 3-year period preceding the study (III, IV). The patients and/or their parents filled out questionnaires on the patient's fracture history, and the fractures were also specially asked about during the study period. For each reported fracture, the localization and mechanism of injury were recorded. Radiographic documentation was obtained from hospital records for all fractures. A high-energy trauma was defined as a fall of >3 m or a traffic accident (I-IV).

In the cross-sectional part of the study, laboratory and anthropometric data, BMD evaluation with IVA images, spinal radiographs, evaluation of pubertal development, and data collection by questionnaires were performed concurrently (I, III, IV). All patients were clinically assessed by two pediatric rheumatologists (I) or by a pediatric nephrologist (III, IV). Global assessment of overall well-being by the parents or guardians, physician's global assessment of disease activity, and Childhood Health Assessment Questionnaire (CHAQ) were administered to all patients with JIA. A 3-day dietary recall was obtained for patients with arthritis, and based on these recordings, the average daily intakes of calcium and vitamin D were calculated for each patient using the computer program AIVO 2000-Diet32, version 1.4.2.1, which is based on the Finnish dietary references for 1998 (I). A detailed clinical examination of the spine and extremities was performed on all transplant patients in Study II by two orthopedic surgeons. Information on the use of dairy products and calcium or vitamin D supplements were obtained by a detailed review in Studies I, III, and IV.

Fracture incidence was assessed also prospectively during a 5-year prospective study period in Study II. Fracture history was systemically recorded at yearly follow-up visits during this study, and a thorough clinical examination of the spine was performed at the beginning and at the end of this prospective part of the study. The development of BMD was also prospectively evaluated during a 2-year period in 14 children with a history of a recent RTx (IV).

4.2.2 Growth assessment

Height was measured with a Harpenden stadiometer and weight in thin underwear with an electric scale; values were compared with Finnish growth charts (Sorva et al. 1990, Pere 2000). Height SD score (height Z-score) was defined as deviation of height, in SD units, from mean height for age and sex. Weight was expressed as a height-adjusted value, as a percentage of the mean ratio in a normal population of the same gender and height, according to Finnish standards (Sorva et al. 1990, Pere 2000). Body mass index (BMI) was calculated as weight in kilograms divided by square of height in meters (kg/m^2) . Pubertal maturation was assessed according to Tanner (1962) as part of the clinical assessment (I, III, IV).

4.2.3 Biochemistry

The following laboratory parameters were assessed in Studies I, III, and IV. Plasma calcium, phosphate, alkaline phosphatase, and creatinine were measured using standard methods. Plasma concentrations of alanine aminotransferase, gamma glutamyltransferase, and total and conjugated bilirubin were analyzed using standard methods (III), and blood urea nitrogen and magnesium were measured (IV). Reference ranges for plasma alkaline phosphatase were age- and sex-dependent, and the measured values were transformed into Z-scores using normal values to allow for cross-sectional comparison. Serum 25-OH-D was assessed by liquid chromatography (Turpeinen et al. 2003), $1,25\text{-}(OH)_2D$ by RIA (only in Study IV), and plasma fasting PTH by an immunoluminometric method. Girls >8 years and boys >10 years were assessed also for serum follicle-stimulating hormone, luteinizing hormone, and estradiol or testosterone. All blood samples were drawn between 7:30 and 10:00. Urine was analyzed for calcium/creatinine ratio in all patients (I, III, IV).

Glomerular filtration rate (GFR) was measured by ^{51}Cr-EDTA clearance, and the measured clearances were corrected for a standard body area of 1.73 m^2 (III, IV).

4.2.4 Imaging studies

4.2.4.1 Bone mineral density measurement

Data on LS BMD measurements, carried out as part of the routine posttransplantation follow-up, were collected from hospital records for each patient (III, IV). In the cross-sectional studies, BMC and aBMD of the LS (L1-L4), femoral neck, total hip, and whole body were measured with DXA (Hologic Discovery

A, pediatric software, version 12.4). All measured values were transformed into Z-scores using the equipment-specific age- and sex-adjusted reference database for the United States Caucasian children. The validity of these reference values was tested in a cohort of 199 healthy Finnish children (125 girls and 74 boys; age range 7.4-18.8 years, median 13.1 years) who were assessed with the same DXA equipment as part of an ongoing study evaluating bone health in healthy schoolchildren in the Helsinki area. Their median BMD Z-scores were 0.0, +0.1, and 0.0 for the LS, total hip, and whole body, respectively, confirming that the use of equipment-specific reference data in this study was justified (I, III, IV). Whole-body lean tissue mass (LTM) and BMC were obtained by DXA. The BMC/LTM ratio was calculated for both height and age according to Högler et al. (2003), and the results were transformed into Z-scores. The BMC/LTM ratio was used to correct the BMC values for body size and muscle mass (I, III).

The aBMD Z-scores were calculated for both calendar age and bone age. The correction for skeletal maturation instead of "height age" was used because bone age was delayed by ≥ 1 year in 24% of JIA patients, 43% of liver transplant patients, and 42% of renal transplant patients. The aBMD Z-scores corrected for bone age were higher than the values calculated for calendar age at all measured sites. Therefore, bone-age-adjusted Z-scores were used in the analysis of the study measurements; only calendar-age-adjusted Z-scores were available for the longitudinal analysis.

4.2.4.2 Radiographic evaluation

Bone age was determined from a plain radiograph of the left hand according to Greulich and Pyle (1959). To detect vertebral compression fractures, anteroposterior and lateral images of the thoracic and LS (IVA) were obtained once for each patient using the DXA scanner (I-IV). If the LS aBMD Z-score was ≤-2.0, if a VFx was suspected in IVA images, or if the vertebrae could not be sufficiently visualized in IVA images, standard spinal radiographs were taken (I, III, IV). Vertebral deformities and compression fractures were assessed according to the classification of Mäkitie et al.(2005). This method was developed for the evaluation of vertebral body morphology changes in chronically ill children with secondary osteoporosis. The vertebral changes were classified as normal (grade 0 or 1), anterior wedge deformities (only the anterior part of the vertebra compressed), or compression deformities (also the middle and/or posterior part of the vertebra are compressed). These abnormal changes were further classified as mild (2a; 20-49% anterior height reduction) or severe (2b; ≥50% anterior height reduction) anterior wedge deformities, or mild (3a; 20-49% mild height reduction) or severe (3b; ≥50% middle height reduction) compression deformities (I-IV).

Posteroanterior and lateral radiographs of the thoracic and LS were obtained at the final follow-up of the prospective part of the study for subjects with a history of VFx, back pain, increased kyphosis, rib hump, or other spinal deformity in the clinical examination. For the remaining patients, these images were obtained by the DXA scanner (II).

4.3 Ethical considerations

The study protocols were approved by the Ethics Committee of the Hospital for Children and Adolescents, University of Helsinki. A written informed consent was obtained from all patients and/or their guardians.

4.4 Statistics

Descriptive data were reported as medians and ranges or as means ± SD. Simple regression analysis was used for correlations, unpaired two-tailed Student's T-test to compare means, and the Mann-Whitney U-test to compare nonnormally distributed variables. Comparisons between three or more groups were made by ANOVA, and for nonparametric data the Kruskall-Wallis test was used. The Chi-square test was applied when nominal data were compared (I, III, IV).

The Kaplan-Meier test was used to estimate the probability of patients remaining free of fractures during the follow-up, and the stratified Kaplan-Meier curves were applied to assess the risk factors for fractures after transplantation (II). Age-and sex-adjusted hazard ratios and 95% confidence intervals for different fracture types in the patients and controls were calculated by Cox's proportional hazard regression model, and a multicovariate Cox's proportional hazard regression model was used to assess the effects of different risk factors for developing any type of fracture in the patient group (II).

Multivariate logistic regression was used to identify and determine odds ratios (ORs) and 95% confidence intervals for significant predictors of low BMD (IV). A p-value of less than 0.05 was considered significant.

5. RESULTS

Bone health was evaluated in 62 (19 males) children and adolescents with JIA who had been treated with GCs and multiple drug combinations and were aged between 4 and 18 years (I). In the epidemiological study, the incidence of fractures was evaluated in a population-based cohort of 196 (121 males) children, adolescents, and young adults with a history of solid organ transplantation before the age of 18 years (II). Bone health evaluation was performed on 40 (21 males) LTx and 106 (65 males) RTx patients aged between 4 and 20 years and who had at least a 6-month follow-up after LTx or RTx. All of these patients were receiving calcineurin inhibitor, antimetabolite, and low- dose MP (III, IV).

The clinical characteristics of the patients are shown in Table 4. Most of the subjects with JIA were of normal stature, while about one-fourth of the children and adolescents after transplantation had a height Z-score below -2.0. Overweight was found in 38% after RTx. The median heights and height-adjusted weights of the LTx and RTx patients in the cross-sectional part of the study are presented in Table 4 and during the longitudinal follow-up in Figure 7. Bone age was delayed by ≥ 1 year in 24% of JIA, 43% of liver, and 42% of renal transplant patients. Since bone age was significantly delayed in such a large proportion of subjects, BMD values were corrected for bone age. The bone age-adjusted BMD values were higher in all of our study groups, and these corrected values, presented in Figure 8, were used in the cross-sectional analysis (I, III, IV).

Table 4. Clinical characteristics of patients in Studies I-IV.

	Study I JIA n = 62	Study II Tx n = 196	Study III LTx n = 40	Study IV RTx n = 106	Controls n = 199
Male gender, n (%)	19 (31)	121 (62)	21 (53)	65 (61)	74 (37)
Age at study time, years	11.8 (4.6-17.9)	15.7 (3.7-30.4)	14.0 (4.1-19.8)	12.6 (4.1-18.8)	13.1 (7.4-18.8)
Age at transplantation, years		6.5 (0.4-18.1)	2.1 (0.4-15.3)	3.4 (0.7-16.4)	
Follow-up after Tx, years		9.2 (2.4-20.5)	7.0 (0.5-15.1)	5.1 (0.5-16.5)	
Age at JIA onset, years	4.0 (1.1-15.3)				
Duration of JIA, years	5.6 (2.0-15.1)				
Height Z-score	+0.1 (-2.9- +1.5)		-1.1 (-4.5- +0.8)	-1.2 (-3.6- +0.9)	+0.4 (-2.3- +3.2)
Height Z-score < -2.0, n (%)	2 (3)		9 (23)	28 (26)	1 (1)
Height Z-score > 0, n (%)	32 (52)		5 (13)	5 (5)	117 (59)
Height-adjusted weight, %	+4 (-17- +40)		+5 (-21- +62)	+9 (-20- +101)	+3 (-25- +92)
Height-adjusted weight > 20%, n (%)	9 (15)		5 (13)	40 (38)	29 (15)

Values are mean (range) II, median (range) I, III, IV. Tx = transplantation, JIA = juvenile idiopathic arthritis, LTx = liver transplantation, RTx = renal transplantation

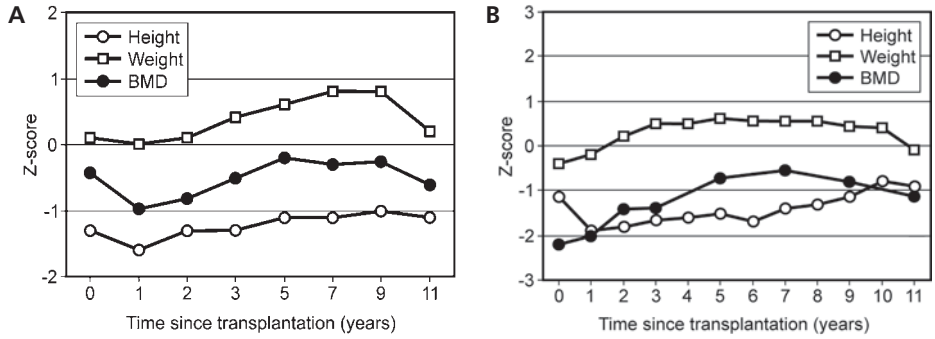

Figure 7. Medians for height, height-adjusted weight, and lumbar spine bone mineral density (BMD) for chronological age during posttransplantation follow-up in 106 children and adolescents with a renal graft (A) and in 40 children and adolescents with a liver transplant (B). Height and BMD are expressed in SD units (Z-score) and weight in percentages.

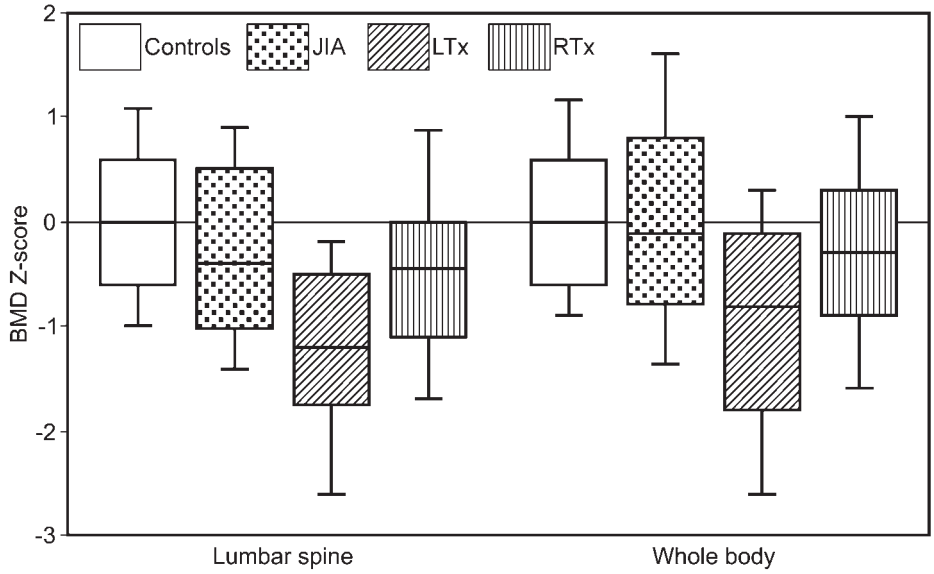

Figure 8. Bone age-adjusted lumbar spine and whole-body BMD Z-scores in controls and patients with juvenile idiopathic arthritis (JIA), liver transplantation (LTx), or renal transplantation (RTx).

5.1 Biochemistry

Median serum vitamin D and PTH of subjects are shown in Table 5 and Figure 9. A vitamin D level below 37 nmol/L was measured in 26% of patients with JIA, and in 13% and 14% of patients with a liver or renal graft, respectively (Table 5). When the vitamin D samples obtained in winter (from October to March) and in summer (from April to September) were analyzed separately, no seasonal variation was observed in any of the patient groups. The lowest PTH values were found in patients with JIA and the highest in the RTx group. None of the patients with JIA or a history of LTx had PTH values above 100 ng/L, while in the RTx patients PTH was above 100 ng/L in 25% of patients (Table 5). A vitamin D value ≥ 80 nmol/L was measured in 3% of JIA patients, 28% of LTx patients, and 25% of RTx patients. Serum calcitriol was within the normal range (48-110 pmol/L) in most of the patients; nine (8%) of the RTx patients had subnormal values (IV).

Most (83%) of the LTx patients were on vitamin D substitution, and 71% of the RTx patients had either vitamin D or alfacalcidol substitution, but only 32% of the JIA patients were on vitamin D substitution (Table 5).

Table 5. PTH and vitamin D values in patients with a history of juvenile idiopathic arthritis (JIA), liver transplantation (LTx), or renal transplantation (RTx).

	Study I JIA n = 62	Study III LTx n = 40	Study IV RTx n = 106
Vitamin 25-OH-D nmol/L, median (range)	49 (13-87)	61 (17-121)	60 (15-166)
Vitamin 25-OH-D < 37 nmol/L, n (%)	16 (26)	5 (13)	15 (14)
Vitamin 25-OH-D ≥ 80 nmol/L, n (%)	2 (3)	11 (28)	26 (25)
PTH ng/L, median (range)	32 (12-38)	43 (16-96)	69 (13-558)
PTH > 100 ng/L, n(%)	0	0	26 (25)
Patients on vitamin D substitution, n (%)	20 (32)	33 (83)	53 (50)
Patients on alfacalcidol, n (%)	0	0	22 (21)

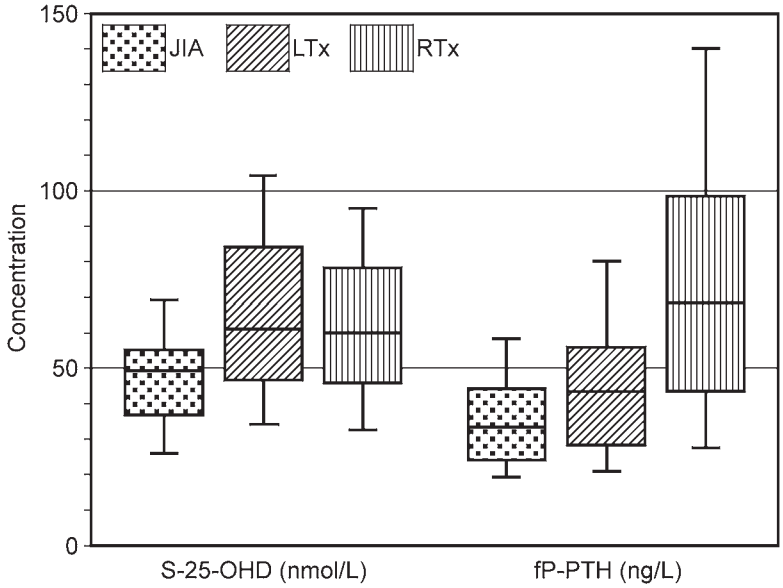

Figure 9. Serum vitamin D and PTH concentrations in patients with juvenile idiopathic arthritis (JIA), liver transplantation (LTx), or renal transplantation (RTx).

5.2 Fractures

5.2.1 Nonvertebral fractures

Four (6%) of the JIA patients had sustained a nonvertebral fracture after the onset of JIA (I). The proportion of Tx patients with nonvertebral fractures was highest in subjects in the epidemiological study (II) and lowest in the RTx patients, 19% and 9%, respectively (II, IV). Five (13%) of the LTx patients had a nonvertebral fracture history (Table 6). The annual incidence of nonvertebral fractures was 3.8% after solid organ transplantation performed during childhood or adolescence and threefold higher in the study group than in the control population (II). No correlation was found between aBMD values or weight-adjusted cumulative GC doses and peripheral fractures in patients with JIA or renal transplantation (I, IV). In LTx patients, the MP exposure was lower in patients with peripheral fractures ($p = 0.018$) (III).

Table 6. Nonvertebral fractures and their localization in study subjects.

	Study I JIA	Study II Epidemiological	Study III LTx	Study IV RTx
Number of patients	62	196	40	106
Number and proportion (%) of patients with fracture	4 (6)	38 (19)	5 (13)	10 (9)
Total number of fractures	4	64	10	12
Localization of fractures				
Upper extremity		37	9	7
Humeral		2	1	
Antebrachium	1	20	4	6
Other	2	15	4	1
Lower extremity		27	1	5
Femoral/pelvic		3	1	
Tibial/ankle		11		1
Other	1	13		4

Table 7. Vertebral fractures in study subjects

	Study I JIA	Study II Epidemiological	Study III LTx	Study IV Rtx
Number of patients	62	196	40	106
Number and per cent of patients with VFx (%)	6 (10)	37 (19)	7 (18)	8 (8)
Total number of fractured vertebrae	14	102	19	17
Symptomatic patients, n (%)	0 (0)	18 (50)	0 (0)	2 (25)

VFx = vertebral fracture

5.2.2 Vertebral fractures

The number and localization of VFx are presented in Table 7 and Figure 10. In Study II, half of the patients with VFx had symptoms, e.g. back pain, and 11% needed operative fracture treatment. In the other studies (I, III, IV), most of the patients reported no pain, and none required operative treatment. Most of the fractures were detected in the thoracic spine (Figure 10).

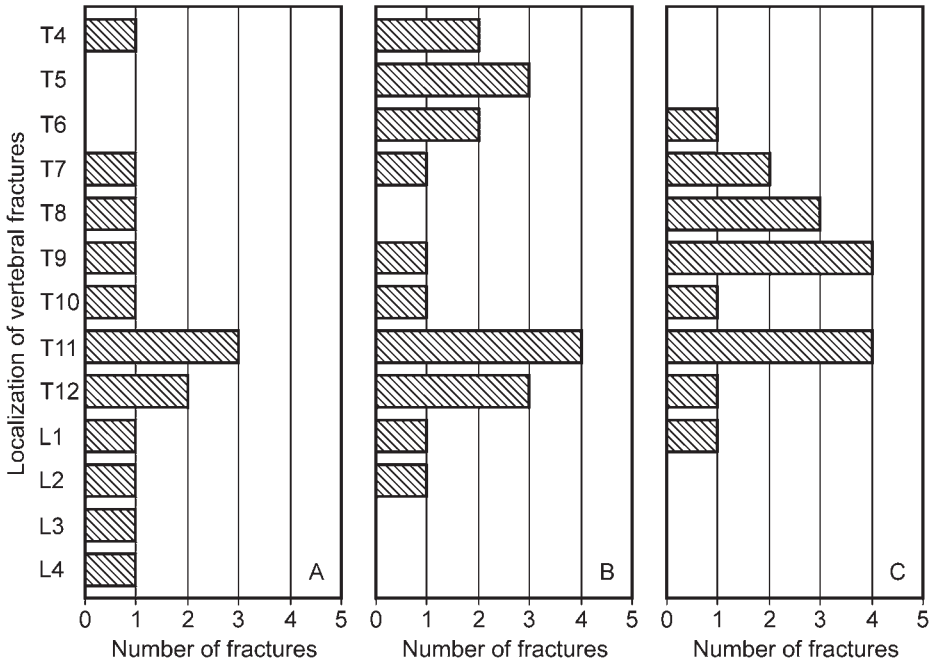

Figure 10. Number and localization of vertebral fractures in 62 patients with JIA (A), 40 patients with liver transplantation (B), and 106 with renal transplantation (C).

In patients with arthritis, the duration of GC treatment or weight-adjusted cumulative GC dose did not differ in those with VFx compared with other study subjects, and none of the patients with VFx had bone age-adjusted aBMD Z-scores below -2.0. No gender difference was found in the prevalence of VFx.

In the epidemiological study of fracture incidence, older age at the time of transplantation, higher BMI, male gender, LTx, and a history of nonvertebral fracture prior to transplantation increased the risk for VFx, while the type of graft, retransplantation, or number of acute rejections were not associated with the risk of VFx. The age, and sex-adjusted hazard ratio with 95% confidence interval for VFx was 61.3 (CI 40.7-92.4). The average weight-adjusted (mg/kg) maintenance doses of CsA, Aza, or MP did not correlate with vertebral fractures (II).

The LTx subjects (III) with fractures were significantly older at the time of Tx ($p = 0.002$), more recently transplanted ($p = 0.019$), had higher BMI ($p = 0.029$) and whole-body fat percentage ($p = 0.025$), and lower aBMD Z-scores at the LS ($p = <0.001$), femoral head ($p = 0.048$), and whole body ($p = 0.030$) than those without VFx (Table 8). No difference was found in MP exposure. Five of the seven patients with VFx had LS BMD Z-score $\leq$ -2.0. No differences were

observed in the laboratory parameters. All patients with VFx were adolescents (age range 12.0-17.3 years), and five of the seven had undergone transplantation after the age of 10 years.

The BMD Z-scores in RTx patients with VFx did not differ from the values of nonfractured patients (IV). The patients with VFx were older at the time of the study and had longer time since renal transplantation, but their age at transplantation did not differ from the others (Table 8). Nor did laboratory parameters, measured GFR, or weight-adjusted MP dose during the preceding three years or the time on dialysis differ from the others.

Table 8. Comparisons of 40 pediatric liver and 106 renal transplant recipients with vertebral fractures.

Characteristic	Vertebral fractures after LTx			Vertebral fractures after RTx		
	yes n = 7	no n = 32	p-value	yes n = 8	no n = 98	p-value
Male gender	3	18	ns	6	59	ns
Age at study, years	14.7 ± 2.0	12.2 ± 4.8	ns	14.8 ± 1.8	11.5 ± 4.5	0.037
Age at transplantation, years	11.0 ± 4.7	3.6 ± 3.9	0.002	4.7 ± 4.6	5.4 ± 4.6	ns
Time since transplantation, years	3.7 ± 3.8	8.5 ± 4.9	0.019	10.2 ± 4.9	6.2 ± 4.6	0.019
Height Z-score	-0.7 ± 1.1	-1.4 ± 1.3	ns	-1.0 ± 0.8	-1.4 ± 1.0	ns
Height-adjusted weight %	15.4 ± 16.7	6.4 ± 17.6	ns	13.9 ± 20.5	14.7 ± 23.9	ns
BMI	22.8 ± 5.9	18.5 ± 3.5	0.029	20.6 ± 3.3	19.5 ± 4.4	ns
Body fat (%)	35.9 ± 9.1	26.0 ± 9.9	0.025	22.8 ± 11.8	26.8 ± 9.9	ns
Lumbar spine BMD Z-score*	-2.4 ± 0.8	-1.0 ± 0.8	< 0.001	+0.2 ± 0.8	-0.5 ± 1.0	ns
Hip BMD Z-score	-1.6 ± 1.0	-0.8 ± 0.9	0.048	+0.1 ± 0.9	-0.3 ± 1.2	ns
Whole-body BMD Z-score	-1.8 ± 1.2	-0.7 ± 0.9	0.030	-0.1 ± 0.7	-0.3 ± 1.1	ns
Duration of MP treatment, days	1878 ± 1574	3134 ± 1782	ns			
MP exposure[mg/kg]/days)	0.19 ± 0.11	0.19 ± 0.15	ns			
Cumulative weight-adjusted MP dose, mg/kg				84 ± 28	113 ± 67	ns
fP-PTH , ng/L	34 ± 18	48 ± 21	ns	85 ± 40	83 ± 76	ns
S-25-OH-D, nmol/L	70 ± 22	64 ± 27	ns	74 ± 16	63 ± 26	ns
GFR, ml/min/1.73 m²	70 ± 20	83 ± 30	ns	53 ± 30	58 ± 21	ns

All values are mean ± SD

BMD values are corrected for bone age, LTx = liver transplantation, RTx = renal transplantation, MP = methylprednisolone

PTH = parathyroid hormone, S-25-OH-D = 25 hydroxyvitamin D, GFR = glomerular filtration rate

* 1 patient in both group with VFx in the lumbar spine was excluded from the analysis

5.3 Bone mineral density

5.3.1 Retrospective analysis of bone mineral density development

Altogether 208 and 478 lumbar spine BMD assessments were available for the longitudinal analysis of BMD in 40 LTx patients and 106 RTx patients, respectively (III, IV). The median LS BMD Z-score was -2.2 at the time of transplantation and ≤ -2.0 in 75% of the LTx patients (III). It then gradually increased during the following years to a peak value of -0.5 at seven years (Figure 7 B) posttransplantation when the Z-score was ≤ -2.0 in 22% of the subjects. Complete BMD data for the pubertal years were available for 10 LTx patients. In eight of these the aBMD Z-score decreased by ≥ 0.7 during puberty and was significantly subnormal (mean LS Z-score -1.9, range -0.8- -4.4) at completion of puberty in all of them (Figure 11 A, B).

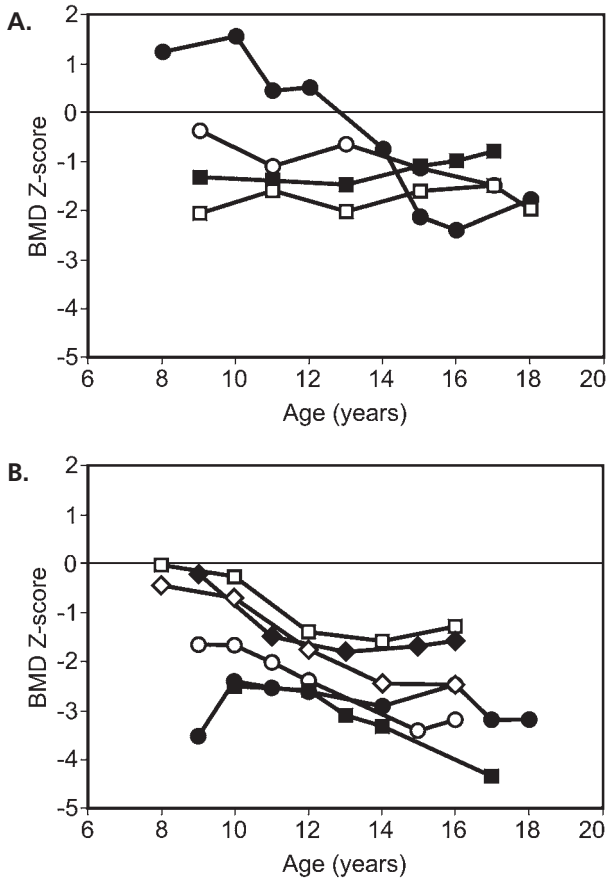

Figure 11. Development of lumbar spine BMD Z-score over puberty in four females (A) and six males (B) after LTx.

In RTx patients, the median LS BMD Z-score was -0.4 at the time of RTx and ≤ -2.0 in 9% of the subjects. It decreased postoperatively (median Z-score -1.0 at year one), but increased during the following years to attain a peak value of -0.2 at five years postoperatively (Figure 7 A). During puberty the median aBMD Z-scores increased in male subjects, in contrast to females, for whom the scores decreased (Figure 12). Concurrently, the median height Z-score remained at -1.0 nine years after RTx despite catch-up growth (Figure 7 A).

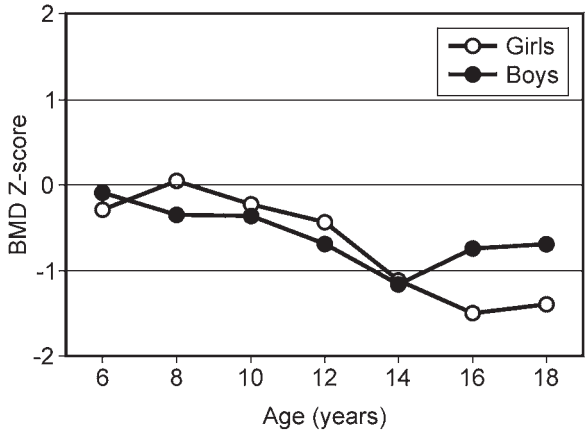

Figure 12. Development of lumbar spine BMD Z-score over puberty in girls and boys with RTx.The curves represent median values.

5.3.2 Cross-sectional analysis

A comprehensive aBMD analysis with DXA and clinical examination were performed on JIA, LTx, and RTx patients. The median aBMD values for the LS and whole body were higher when corrected for bone age. The median LS BMD Z-scores were -0.4, -1.2, and -0.5, and the median whole-body BMD Z-scores were -0.1, -0.8, and -0.3 in the patients with JIA, LTx, and RTx, respectively. The LS BMD Z-scores were ≤ -2.0 in three of the JIA patients, five of the LTx patients, and four of the RTx patients and for the whole body in one, six, and four patients, respectively (I, III, IV) (Table 9).

Table 9. Bone mineral density in children with juvenile idiopathic arthritis, liver, or renal transplantation

	Study I JIA n = 62	Study III LTx n = 40	Study IV RTx n = 106
Lumbar spine aBMD Z-score	-0.4 (-2.8 - +1.8)	-1.2 (-3.8 - +0.8)	-0.5 (-2.8- +2.4)
Lumbar spine Z-score ≤ -2.0, n (%)	3 (5)	6 (15)	4 (4)
Hip aBMD Z-score	-0.1 (-2.1 - +2.4)	-0.9 (-2.6- +0.9)	-0.2 (-3.0- +2.9)
Hip aBMD Z-score ≤ -2.0, n (%)	1 (2)	5 (13)	6 (6)
Whole-body aBMD Z-score	-0.1 (-1.9 - +2.4)	-0.8 (-3.6 - +0.6)	-0.3 (-3.2- +1.8)
Whole-body aBMD Z-score ≤ -2.0, n (%)	1 (2)	6 (16)	4 (4)

5.3.3 Prospective analysis

A prospective more thorough evaluation of BMD development during the first 2 years after RTx was performed in 14 patients. It showed a decrease in BMD at 6 months after RTx in all 8 male subjects and in 4 of the 6 female subjects. Thereafter the BMD values recovered.

5.4 Determinants of bone mineral density

We studied various parameters in relation to BMD to identify the clinical and treatment-related factors that may predispose to impaired BMD in patients with JIA or a solid organ transplant.

5.4.1 Age and duration of dialysis treatment

No correlation was found between the age at onset of JIA and aBMD Z-scores (I). Children who had undergone LTx before the age of two years had statistically higher aBMD Z-scores at all measured sites than those who were transplanted at an older age. However, the BMD values of children with RTx below two years of age did not differ from the other subjects (Table 10). Similarly, if the LTx was performed below the age of 10 years, the patients had higher BMD Z-scores for LS, hip, and whole body, with p-values of 0.005, 0.007, and 0.006, respectively. The six patients with LS Z-score ≤ -2.0 were transplanted at an older age (14.1 vs. 2.0 years, p = 0.002) (III). Seventeen patients (16%) on dialysis for more than two years before RTx had lower LS aBMD Z-scores (IV).

Table 10. Clinical characteristics and bone mineral density parameters in children and adolescents after liver or renal transplantation at the age of ≤ 2 years.

	Age at LTx			Age at RTx		
	≤ 2 years	> 2 years	p-value	≤ 2 years	> 2 years	p-value
	n = 19	n = 21		n = 36	n = 70	
Age at study time, years	10.9 ± 4.0	14.2 ± 4.5	0.011	9.8 ± 4.1	12.7 ± 4.2	ns
Follow-up after transplantation, years	9.6 ± 4.0	6.0 ± 5.3	0.024	8.4 ± 4.0	5.4 ± 4.7	0.002
Height Z-score	-1.2 ± 1.2	-1.3 ± 1.4	ns	-1.2 ± 1.0	-1.4 ± 0.9	ns
Height-adjusted weight, %	+6.6 ± 14.3	+9.2 ± 20.4	ns	+8.4 ± 13.6	+17.8 ± 26.9	ns
aBMD Z-score for lumbar spine	-0.7 ± 0.6	-1.7 ± 0.9	0.002	-0.5 ± 0.9	-0.4 ± 1.0	ns
aBMD Z-score for hip	-0.5 ± 0.8	-1.3 ± 0.9	0.004	-0.3 ± 1.0	-0.2 ± 1.2	ns
aBMD Z-score for whole body	-0.5 ± 0.8	-1.3 ± 1.1	0.030	-0.1 ± 1.0	-0.4 ± 1.0	ns

All values are mean ± SD. BMD Z-scores are corrected for bone age

5.4.2 Renal function

All of the patients with JIA had normal plasma creatinine, and it showed no correlation with aBMD values (I). The measured GFR was above 90 mL/min/1.73 m^2 in 13 (33%), between 60 and 90 mL/min/1.73 m^2 in 18 (45%), and between 30 and 59 mL/min/1.73 m^2 in 9 LTx patients (22%) (III). In RTx patients, GFR was above 90 mL/min in 10 (9%), between 60 and 90 mL/min in 35 (33%), between 30 and 59 mL/min in 52 (49%), and below 30 mL/min in 9 subjects (9%). None of the patients were on dialysis (IV). No statistical difference was found between these groups in aBMD values at any measured site (III, IV).

5.4.3 Vitamin D and parathyroid hormone

Twenty-six (25%) of the RTx patients had a 25-OH-D value ≥ 80 nmol/L. Their aBMD Z-scores for the hip were higher than in the other RTx subjects (+0.3 vs. –0.4, p = 0.0023). Fifteen subjects (14%) had a vitamin D value below 37 nmol/L, and they had lower aBMD Z-scores for the hip and whole body, -0.9 vs. -0.2, p = 0.019, and -1.0 vs. -0.1, p = 0.002, respectively (IV). In a simple regression analysis, no correlation was found between PTH or vitamin D values and aBMD Z-scores in JIA or liver transplant patients (I, III). In Study I, the JIA patients with vitamin D values below 37 nmol/L had statistically higher PTH values (45 vs. 33, p = 0.007). No correlation was present between serum 1,25 $(OH)_2D$ and aBMD values (IV).

5.4.4 Medication

The total duration of GC treatment or weight-adjusted cumulative GC dose in patients with JIA showed no correlation with aBMD values in any measured site. JIA patients treated with TNF-α antagonists had significantly higher mean duration (1993 days vs. 734 days, p = < 0.001) and mean cumulative weight-adjusted dose (387 mg/kg vs. 99 mg/kg, p = < 0.001) of GC than patients not taking TNF-α antagonists, but their aBMD values did not differ from the values of other JIA patients. The aBMD values of patients who had been on GC treatment during the year preceding the study did not differ from the others (I).

The cumulative MP exposure (mg/kg/day) during the total posttransplantation period after LTx did not correlate with aBMD values in any measured site (III). Also when the 36 patients with less than three years since RTx were excluded, no correlation was found between cumulative weight-adjusted MP dose and aBMD values in the remaining 70 subjects (IV).

5.4.5 Predictors for low bone mineral density in logistic regression analyses

Logistic regression analysis was performed to evaluate possible predictors for aBMD Z-scores ≤ -1.0 at the LS or hip in RTx patients (IV, Table 11).

Gender alone was not a significant predictor, but when combined with patient's age at study assessment it was highly significant. The OR for the LS and hip aBMD ≤-1.0 in girls over 15 years were 56.26 (95% CI from 5.17 to 611.82) and 13.47 (95% CI from 1.17 to 154.69), respectively. When comparing the 9 girls and 22 boys aged >15 years with each other, no difference was observed in the duration of dialysis, age at RTx, time since RTx, height Z-score, height-adjusted weight, or GFR. A PTH value over 100 ng/L at the time of the study was a significant independent predictor for a low LS aBMD Z-score, with OR 4.03 (95% CI from 1.37 to 11.85), and similarly, vitamin D value below 40 nmol/L at the study time for a low hip aBMD, with OR 11.62 (95% CI from 3.31 to 40.86). Cumulative weight-adjusted MP dose over 150 mg/kg during the preceding three years was an independent risk factor for aBMD Z-score ≤ -1.0 in the LS, with OR 5.72 (95% CI from 1.52 to 21.56).

In the logistic regression analysis, duration of dialysis treatment, age at transplantation, time since transplantation, GFR, and vertebral or nonvertebral fractures were not significant predictors for BMD.

Table 11. Odds ratios and 95% confidence intervals for factors predicting lumbar spine and hip BMD Z-score ≤ -1.0 in pediatric renal transplant patients.

Risk factor	BMD Z-score for lumbar spine ≤ -1.0 n = 31	BMD Z-score for hip ≤ -1.0 n = 22
Age at time of study > 15 years + female gender n = 9	56.26 (5.17 - 611.82) p = 0.0007	13.47 (1.17 - 154.69) p = 0.033
fP-PTH > 100 ng/L n = 26	4.03 (1.37 - 11.85) p = 0.009	1.94 (0.51- 7.35) p = 0.317
S-D-25 < 40 nmol/L n = 18	2.31 (0.60 - 8.95) p = 0.215	11.62 (3.31 - 40.86) p = 0.0001
Cumulative weight-adjusted MP dose during previous 3 years > 150 mg/kg n = 15	5.72 (1.52 - 21.56) p = 0.008	1.87 (0.31 - 11.12) p = 0.484

Logistic regression model was used for analysis
PTH = parathyroid hormone, S-D-25 = 25-hydroxyvitamin D, MP = methylprednisolone

6. DISCUSSION

JIA and solid organ transplantation have been associated with changes in bone metabolism. Several factors may lead to impaired bone health in children with JIA or a history of Tx. Chronic inflammation, GCs, and limited physical activity in patients with JIA, and pre-existing bone disease, GCs, calcineurin inhibitors, impaired renal function, and changes in vitamin D metabolism in Tx patients are thought to have an impact on bone health. We investigated the incidence and prevalence of fractures, distribution of BMD, and vitamin D status in pediatric patients with GC exposure and attempted to identify clinical and treatment-related factors that may negatively affect bone health in these subjects.

6.1 Bone health characteristics in patients with juvenile idiopathic arthritis

Previous studies have reported a high prevalence of osteoporosis in children and adolescents with JIA. The last decade has brought new treatment modalities to children with JIA, but how these medications impact overall skeletal health is unknown. In this study, we evaluated bone health in 62 children with JIA.

The clinical course of JIA is variable; in some patients, the disease remains active for a long time and these individuals may enter adulthood with active disease (Ravelli 2004). Several cross-sectional long-term follow-up studies have reported reduced BMD in adults with a history of JIA (Zak et al. 1999, French et al. 2002). Zak et al. (1999) evaluated 65 adult patients (mean age 32 years) with a history of JIA and found osteopenic (T-score < -1.0) LS and hip aBMD values in 43% and 53% of the subjects, respectively. In the study by French et al. (2002), 28% of adult patients with JIA were osteopenic. Low aBMD values in early adulthood may predispose to increased risk and early presentation of fragility fractures.

Several factors may negatively affect bone health in patients with JIA. Chronic inflammation may result in generalized or periarticular bone loss (Haugeberg et al. 2003, Strand and Kavanaugh 2004). Cytokines and growth factors produced by synovial tissue may increase osteoclast formation, activity, and/or survival, and thus increase bone resorption (Gravallese 2002). Limited physical activity and reduced muscle forces acting on bone may pose an additional risk for osteoporosis (Häkkinen et al. 2004, Lindehammar and Lindvall 2004, Burnham et al. 2006b). However, medications, especially GCs, have been regarded as the main threat to skeletal health in patients with JIA. Both systemically and intra-articularly administered GCs can reduce bone formation and increase its resorption (Canalis 2003). MTX has been reported to cause osteopenia when used to

treat childhood malignancies (Mandel et al. 2004). The dosing in JIA is substantially lower and does not seem to have adverse effects on bone (Cranney et al. 2001, Cimaz 2002). Furthermore, both calcium and vitamin D intake may be suboptimal, and delayed puberty, which is common in patients with JIA, may negatively affect bone mass accrual (Rusconi et al. 2003, Hillman et al. 2008).

In our patients with JIA, the median bone-age-adjusted aBMD Z-scores were -0.4, -0.1, and -0.1 for the LS, hip, and whole body, respectively. Three (5%) of the patients had aBMD Z-score ≤ -2.0 for the LS, one (1%) for the hip, and one (2%) for the whole body. The observed aBMD values were higher than reported in previous studies, but below the values of healthy schoolchildren in Finland, especially in the lumbar region. Most of our patients had normal stature. While all of the patients were on anti-rheumatic drug treatment (MTX, GC, TNF-α-antagonist, hydroxychloroquine, or sulfasalazine), a large proportion (55%) were in clinical remission. The majority (90%) of the subjects were able to participate in normal physical education at school. The positive effect of physical training on bone health is widely recognized and may partly explain the satisfactory aBMD values.

The proportion of patients with a history of nonvertebral fracture did not differ from that of the normal pediatric population. Six (10%) of our patients had, however, asymptomatic VFx. This number is high, particularly in view of the satisfactory aBMD values. This emphasizes the importance of active screening for VFx. DXA alone is not sufficient in detecting VFx, as none of our patients with VFx had an aBMD Z-score ≤ -2.0. Therefore, spinal imaging is also needed for adequate fracture evaluation.

Neither the total duration nor the cumulative weight-adjusted dose of GC correlated with aBMD values or fractures. These results are in line with some previous reports that show low aBMD values even in JIA patients without GC exposure (Lien et al. 2003). In our subjects, no difference in bone health was observed between those patients receiving GC treatment during the survey and those who had previously been exposed to GC.

TNF-α is known to be an important mediator in various inflammatory conditions and in postmenopausal osteoporosis. TNF-α directly inhibits osteoblast differentation, function, and survival and promotes osteoclastogenesis (Feng 2005). TNF-α antagonists have provided a new effective treatment modality also in pediatric patients with arthritis. In addition, they may prevent bone loss directly by regulating osteoclast formation and activation or by decreasing the amount of circulating cytokines (Saidenberg-Kermanac'h et al. 2004, Feng 2005), and indirectly by allowing reduced GC dosing and improved physical activity (Lahdenne et al. 2003, Strand and Kavanaugh 2004, Simonini et al. 2005). During anti-TNF therapy soluble bone turnover markers decrease significantly and aBMD values significantly increase in adult patients with rheumatoid ar-

thritis (Lange et al. 2005, Seriolo et al. 2006, Torikai et al. 2006). Twenty (32%) of our patients had been on TNF-α antagonists. This may partly explain the better aBMD values in our subjects. However, the small number of patients and the lack of prospective data in our study do not allow any firm conclusions to be made. Further longitudinal studies in pediatric patients are needed to elucidate the potential skeletal benefits of anti-TNF therapy.

6.2 Bone health after solid organ transplantation

Transplantation has become an established treatment modality for several diseases, including acute and chronic liver failure, end-stage renal disease, and heart failure. The number of people living with a graft has increased steadily during the last two decades. While the overall survival has improved, new problems, such as osteoporosis and fragility fractures, have emerged. The present epidemiological study showed a high incidence of fractures in 196 subjects who had undergone Tx during childhood or adolescence.

Because the overall incidence of fractures in the adult studies was high and no previous prospective studies were published on fracture incidence in pediatric Tx patients, we performed a retrospective and longitudinal study to define the fracture incidence in Finnish solid organ transplant recipients. Our study comprised 196 children, adolescents, and young adults with a kidney, liver, or heart transplant at or before 18 years of age. Over one third of these patients had sustained one or more fractures postoperatively, and 61% of the 166 fractures were vertebral. The annual incidence for vertebral and nonvertebral fractures postoperatively was 5.7%, and 3.8%, respectively. The incidence was 160-fold higher for the VFx and threefold higher for the non-vertebral fractures as compared with the control population. The number of vertebral and non-vertebral fractures in this epidemiological study was higher than in our subsequent studies on younger patient cohorts with a history of LTx or RTx. This may be due to a longer follow-up time and a larger proportion of patients who had already passed their adolescent growth spurt, period of the highest fracture incidence also in healthy children (Bailey et al. 1999).

In our patient group, one-half of the VFx were asymptomatic. This signifies that if VFx are not specifically searched for, many of them will elude proper diagnosis and treatment. Eight (11%) of the patients with VFx had needed operative treatment. This is a high proportion compared with the observations in our subsequent studies on pediatric LTx and RTx patients; none of them were operated. Higher GC doses were used in the oldest patients. The control of secondary hyperparathyroidism in patients on dialysis has also changed within the last decade. These factors may partly explain the different fracture patterns in the studies. However, this study showed that pediatric patients are also prone to fractures, and we therefore assessed bone health separately in children and adolescents after LTx or RTx.

6.3 Bone health in children and adolescents after liver transplantation

Surgical techniques, intensive care treatment, and immunosuppressive medication have improved greatly during the last 20 years, and today LTx is considered to be a standard care for children and adolescents with terminal liver failure, metabolic disease, or malignancy, with a five-year patient survival rate varying from 70% to 85% (Kayler et al. 2002, Diem et al. 2003). The number of children living with a graft is on the rise, and complications independent of graft function are of increasing relevance to the long-term outcome of these patients. These include psychomotoric and cognitive development, kidney function, and growth and orthopedic problems in these patients (Adeback et al. 2003, Qvist et al. 2003, Campbell et al. 2006, Helenius et al. 2006a, 2006b). Determinants of LTx bone disease are not clearly defined. Pre-existing bone disease, life-long immunosuppressive medication, especially GCs, and disturbances in pubertal development may be important factors. We assessed bone health and its determinants in 40 children and adolescents who had undergone LTx before the age of 16 years.

Okajima et al. (2003) showed in 30 consecutive children who underwent living-related LTx that the LS aBMD Z-scores improved markedly after Tx, with the mean Z-score being + 0.16 after two years. None of these children had fractures postoperatively, although three fractures were detected preoperatively. In contrast to the rapid bone loss reported in adults, no reduction in BMD occurred in the early postoperative period in these patients (Okajima et al. 2003). Khan et al. (2006) reported cross-sectional bone health data on 15 young adults who had undergone LTx during childhood or adolescence. The mean time after LTx was 12 years. Four limb fractures were reported postoperatively in these patients, but VFx were not assessed. The mean BMD Z-score for the LS was -0.4 and for the whole body -0.9; BMD values were statistically higher in patients on GCs at the time of the study (Khan et al. 2006).

VFx were present in 18% of our LTx patients. The fractures seemed to occur quite soon after LTx, especially in adolescent patients. The higher prevalence of VFx in recently transplanted patients suggests that the high GC doses used immediately after Tx may be more harmful than the cumulative dose and total duration of GC treatment. All of the VFx were asymptomatic, and the exact time of their occurrence remained unclear. Spontaneous remodeling after VFx occurs in early childhood, but usually not after the age of 13 years (Karlsson et al. 2003). Since BMD values significantly improved during the postoperative years, some recovery in vertebral morphology likely also occurred. The aBMD Z-scores were significantly lower in patients with VFx, in all measured sites. Almost all of the observed VFx were located in the thoracic region and only 11% in the lumbar region. In adults, vertebral fractures in the LS are more

common. Gallacher et al. (2007) reported that 22% of the subjects had fractures in the lumbar region and 21% in both the thoracic region and the LS. Therefore, the thoracic spine should be carefully evaluated for the presence of fractures, and standard spinal radiographs are often needed because thoracic vertebrae do not clearly visualize in the images produced by DXA (Mäyränpää et al. 2007).

In the retrospective analysis, we found that the LS BMD was significantly reduced at the time of LTx, but gradually improved to a peak value of -0.5 at seven years postoperatively. These findings are in line with previous studies. However, the median BMD remained subnormal and at a lower level than earlier reported in children (Okajima et al. 2003). Bone loss after LTx is greatest during the first 3-6 months postoperatively, when the GC doses are highest; this has been confirmed by bone histomorphometry (Bjoro et al. 2003, Guichelaar et al. 2004, Guichelaar et al. 2006).

The cross-sectional analysis in which the BMD values were corrected for bone age showed that more than half of our patients had LS Z-score ≤ -1.0 and 15% had ≤ -2.0, indicating that low BMD values are common in pediatric LTx recipients. The 19 patients (48%) transplanted as infants had higher aBMD values for the LS, hip, and whole body than those who were operated on later. The results suggest that low-dose GC exposure, even over several years, is usually well tolerated and does not necessarily translate into severe skeletal morbidity in children. The six patients with LS aBMD Z-score ≤ -2.0 were all transplanted at an older age (14.1 vs. 2.0 years, $p = 0.002$).

Complete data on BMD development during pubertal years was available for 10 of our subjects. In eight patients (80%), the aBMD Z-score decreased by ≥ 0.7 during puberty, remaining significantly subnormal (mean LS Z-score -1.9) at the end of puberty in all of them. However, the median height Z-score remained stable during puberty signifying normal timing of the pubertal growth spurt. In healthy children, the most rapid increase in aBMD occurs during puberty in both genders, at 11-13 years in girls and at 13-17 years in boys (Bonjour et al. 1991). Individual sensitivity to GCs is a recognized modifying factor and the patient's age may also play a role, as pubertal patients seemed to be more prone to skeletal complications. Even small doses of GCs might interfere with normal bone mass accrual during the critical time of bone mass gain by negatively influencing the synthesis and action of gonadotrophins and sex steroids (Canalis and Giustina 2001).

6.4 Bone health in children and adolescents after renal transplantation

The frequency of low BMD values in children is dependent on the interpretation of results (Leonard 2005). In general, correction of BMD values for statural height or weight (instead of chronological age) reduces the frequency of subnormal values in children and adolescents (Klaus et al. 1998, Sanchez et al. 1998 Saland et al. 2001). Children with chronic renal failure often have short stature and linear growth is impaired even after successful RTx. Therefore, this correction of BMD values is important (Offner et al. 1999, Ninik et al. 2002, Nissel et al. 2004). In our patients, bone age was delayed by more than one year in almost half of the patients. We found thus it important to correct the BMD values for bone age to avoid overestimation of the BMD reductions in the cohort.

We evaluated bone health and the prevalence of low BMD, fractures, and longitudinal changes in BMD in a cohort of 106 pediatric RTx patiens. The overall bone health in our pediatric RTx patients was satisfactory. The bone-age-corrected BMD values were normal in most RTx patients, and the Z-score for LS, hip, and whole body was below –2.0 in only 4-6% of patients. Only one previous study has been published in which correction for bone age was used in 19 renal transplant children. In that study, the BMD Z-score for LS was below -2.0 in 63% vs. 26% and for femoral neck in 56% vs. 17% of patients when the values were adjusted for patient's chronological age or bone age, respectively (Goksen et al. 2005).

In Finland, the proportion of patients who have undergone RTx as infants is higher than in any other transplantation center in the world. This is due to the exceptionally high prevalence of nephrotic syndrome of the Finnish type, a disease belonging to the Finnish disease heritage. We therefore compared the BMD results of those transplanted before the age of two years with the others. Importantly, we showed that the aBMD Z-scores of children and adolescents transplanted as infants did not differ from those who underwent Tx at an older age. The exposure to the low-dose alternate- day GC regimen was longer in these subjects, but this treatment seemed to be well tolerated. The time on dialysis did not differ in these patient groups.

Low BMD values for both the LS and hip were significantly more common in adolescent girls than in younger girls or adolescent boys. This is unlikely due to delayed pubertal development since the mean age of menarche was normal (12.9 years) also in the females who were over 15 years at the time of this study. In adolescent females, even small doses of GCs have been speculated to interfere with bone mass accrual by negatively influencing the synthesis and action of gonadotrophins and sex steroids (Canalis and Giustina 2001).

According to these observations, careful bone health evaluation is warranted, especially during puberty.

In Studies I and III on children with JIA and LTx, we failed to find a correlation between total GC exposure and BMD values; instead of the overall cumulative GC dose, skeletal health may be determined by more recent GC exposure. Therefore, only GC doses used during the three preceding years were included in Study IV. Logistic regression analysis confirmed that the cumulative MP dose during the preceding three years was a significant independent risk factor for low BMD (OR 5.72;1.52 -21.56, p = 0.008). Patients with a higher MP dose had a shorter time since RTx and were exposed to acute rejections, which mostly occur postoperatively during the first six months. Individual sensitivity to GC is a recognized modifying factor, and the patient's age may also play a role, as pubertal girls were more prone to skeletal complications (Chesney et al. 1984, Cooper 2004).

High-turnover bone disease associating with high serum PTH levels is the most common form of renal osteodystrophy in children on dialysis (Wesseling et al. 2008). Usually CKD-bone and mineral disease improves after RTx. However, in some patients hyperparathyroidism may persist for years. During dialysis treatment, oversuppression of PTH should be avoided to prevent development of adynamic bone disease (Wesseling et al. 2008).

The prevalence of VFx in our cohort was 8%, which is high considering the satisfactory BMD values. Most of the fractures were asymptomatic and would not have been detected without specific screening. Some of the patients transplanted as infants might have sustained VFx, but because of the suggested tendency for spontaneous recovery of VFx, during childhood growth (Karlsson et al. 2003) these fractures were no longer visible at the time of this study. Whether such spontaneous recovery also takes place during systemic GC treatment remains unknown.

6.5 Juvenile idiopathic arthritis or solid organ transplantation and vitamin D

Low vitamin D values were found in all of our patient groups exposed to GC treatment. The optimal vitamin D level for risk groups to prevent secondary osteoporosis is speculated to be about 80 nmol/L and only one-fourth of our patients achieved this level (Bischoff-Ferrari et al. 2006). For healthy children and adolescents vitamin D level of > 50 nmol/L is considered as indicative of vitamin D sufficiency (Misra et al. 2008). In contrast to previous studies no seasonal variation of vitamin D values was found in our patients. The use of sunscreens, highly recommended for early prevention of skin cancer in children after Tx, may partly explain this. Also, vitamin D substitution may mask this variation.

Little is known about calcium and vitamin D in JIA. In a study of 13 JIA patients, the mean baseline aBMD Z-score for the LS was -2.8 ± 0.5, and this increased with vitamin D substitution to -2.3 ± 0.5 after six months and to -2.4 ± 0.4 after one year (Reed et al. 1991). In most of our patients, the vitamin D values were suboptimal, and only two (3%) of the subjects reached the level of 80 nmol/L. Only one-third of the patients were on vitamin D substitution. It is important to ensure sufficient intake of these nutrients, as vitamin D deficiency is highly prevalent in healthy children and adolescents, especially in the northern latitudes (Lehtonen-Veromaa et al. 2002, Välimäki et al. 2004).

Eleven (28%) of our LTx patients had vitamin D values ≥ 80 nmol/L, while five subjects (13%) had level below 37 nmol/L. Most of the patients were on vitamin D and calcium substitution. However, based on these results, the dosing may have been inadequate for attaining optimal vitamin D levels. None of the patients had PTH values greater than 100 ng/L.

Plasma PTH above 100 ng/L was an independent predictor for reduced LS BMD in patients with RTx. Cohen et al. (2005) reported in nine adult survivors of pediatric HTx that elevated PTH may contribute to osteoporosis. In another study, all 58 adult RTx patients had elevated PTH values despite good renal function, and higher PTH values were measured in those with osteoporosis and high bone turnover (Cruz et al. 2002). According to the European pediatric guidelines, the PTH levels should be within the normal range in patients not on dialysis (Klaus et al. 2006). Vitamin D deficiency may cause PTH elevation also in patients with good graft function, and thus should be avoided.

6.6 Limitations of the study

Several limitations, mainly related to the study populations and applied methodology, were identified during this study. While the studies on fracture epidemiology in the transplant population and on bone health determinants in the RTx patients included a large number of patients, the studies on JIA and LTx patients were insufficiently powered to assess independent predictors of bone health. Furthermore, the cohort of JIA also included patients with relatively mild disease and may thus have failed to show the spectrum of skeletal complications in patients with more severe presentation. The number of patients with JIA treated with TNF-α antagonists was small, and the potential benefits of these medications for bone health could not be evaluated. However, the transplant cohorts were larger than in previous pediatric studies and included practically all pediatric LTx and RTx patients in the country.

We did not have control groups for most of the cohorts. In the epidemiological study, the control group consisted of patients aged 0-30 years, who had sustained a fracture during 2001 and who were treated for the fracture at primary healthcare centers or university hospitals in the city of Helsinki. Possibly, the true fracture rate in the control population may have been higher, as some milder fractures might have been treated in private clinics, and thus were not included in the register. Furthermore, the controls were not screened for VFx, and therefore the observed VFx rate may be an underestimation.

DXA has many limitations in pediatric use. Usually, the child must be at least four years of age to be able to stay still during the measurements. The association between low BMD and fractures is less established in children than in adults. DXA is based on two-dimensional assessment of three-dimensional skeletal structures and measures only aBMD, instead of volumetric BMD. The reference ranges of DXA are based on healthy children, and subjects with short stature or delayed skeletal maturity may have falsely low BMD values. Correction of the BMD for bone age and/or height is essential to avoid misinterpretation of the results. In the cross-sectional studies, we adjusted all BMD values with bone age. However, in the longitudinal analyses, no bone age films were available and only unadjusted values were used.

New methods for more accurate bone quantitation have been developed. Quantitative computed tomography measures true volumetric bone density, which is not size-dependent, and provides separate measurements for trabecular and cortical BMD. The radiation dose is, however, much higher than in DXA, limiting its use in children and adolescents. Peripheral quantitative computed tomography is more useful for pediatric purposes, and the sites used in the measurements include the distal radius, tibia, and femur. This method allows information to be obtained for both trabecular and cortical bone. Unfortunately, no reference values exist for children.

No follow-up data of vitamin D measurements in our patients were available, and therefore vitamin D status at the onset of JIA and at the time of Tx or fracture remains unknown.

6.7 Future considerations

Many of the research questions presented here would be ideally investigated in prospective study settings with larger patient cohorts. A prospective study on patients with newly diagnosed JIA would be optimal to evaluate BMD development and the incidence of fractures. This study setting, with regular BMD assessment with DXA and peripheral computed tomography would allow more reliable assessment of the potential risk factors and the impact of TNF-α antagonists on bone health. In transplantation patients, the role of GCs in bone mass development can be evaluated accurately only if steroid-free protocols are also widely used in children and adolescents.

This study described the spectrum of bone disease in patient cohorts and identified potential risk factors for low BMD and fractures. However, elucidation of optimal preventive measures and the role of bisphosphonates in the treatment of secondary osteoporosis in these patients requires further studies.

7. CONCLUSIONS

Studies I-IV evaluated bone health in patients with JIA or a history of solid organ transplantation.

The main conclusions were the following:

1) Median BMD values were subnormal in all patient cohorts. The values were highest in patients with JIA and lowest in LTx patients. In the transplant patients, BMD was lowest during the immediate posttransplantation years, increasing subnormally during puberty. Delayed skeletal maturation was common in all patient groups, and therefore, correcting BMD results for bone age was essential.

2) Vertebral fractures were common in all patient groups and were mostly asymptomatic. Their diagnosis is thus dependent on active screening by spinal imaging. Vertebral fractures were most common in LTx patients, with a prevalence of almost 20%.

3) The total cumulative weight-adjusted GC did not correlate with BMD or fractures in patients with JIA or LTx. By contrast, the cumulative weight-adjusted dose of GC during the three preceding years was a significant independent risk factor for low LS BMD in RTx patients.

4) Vitamin D deficiency was common, and only 3% of patients with JIA and about 25% of patients with LTx or RTx were considered to have adequate serum vitamin D levels.

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