From the National Graduate School of Clinical Investigation
and the Pediatric Graduate School
Children's Hospital, Helsinki University Central Hospital
and
Institute of Clinical Medicine
University of Helsinki
Helsinki, Finland

FRACTURES IN CHILDREN: EPIDEMIOLOGY AND ASSOCIATED BONE HEALTH CHARACTERISTICS

Mervi Mäyränpää

ACADEMIC DISSERTATION

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Supervised by

Docent Outi Mäkitie
Children's Hospital
Helsinki University Central Hospital
University of Helsinki

and

Docent Pentti E Kallio
Children's Hospital
Helsinki University Central Hospital
University of Helsinki

Reviewed by

Professor Hannu Aro
Department of Orthopaedic Surgery and Traumatology
Turku University Hospital
University of Turku

and

Docent Kirsti Näntö-Salonen
Department of Pediatrics
Turku University Hospital
University of Turku

Official opponent

Docent Lennart A Landin
Department of Orthopaedics
Skåne University Hospital
University of Lund
Malmö, Sweden

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To my family

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Abstract

Fractures are common in childhood. Incidence varies between geographical areas, and it has been proposed that the fractures in children are increasing. Repeated fractures, and especially vertebral fractures, in children may be a sign of impaired bone health, but it remains unestablished when and how fracture-prone children should be assessed. Bone mineral density (BMD) affects bone strength, and it can be measured with dual-energy X-ray absorptiometry (DXA). However, DXA has limitations in growing children, as have the biochemical markers of bone metabolism. In this work, we studied epidemiology of fractures in children. Special attention was given to those children with frequent fractures or vertebral fractures; their bone health was thoroughly assessed. To evaluate the clinical use of two rarely used methods in children, we assessed the accuracy and advantages of vertebral fracture assessment (VFA) by densitometry, and histomorphometry from bone biopsy in children with suspected osteoporosis.

We conducted a prospective study to assess population-based fracture incidence and pattern in children under 16 years, living in Helsinki. Data were gathered from public health care institutions for a 12-month period in 2005. Patients with a significant fracture history and aged 4 to 16 years were further evaluated for skeletal characteristics and predisposing factors at Children's Hospital. Fracture history was regarded as significant if the child had sustained a vertebral fracture, or repeated long-bone fractures (2 fractures before age 10 years or at least 3 fractures before age 16 years) resulting from low-energy trauma. Skeletal health was evaluated with DXA, biochemistry, and radiographs, and life-style factor data were collected by interview; age- and sex-matched controls were used to assess predisposing factors. The accuracy of VFA, the visibility and detection rate of compressed vertebrae, was assessed in 65 children; standard radiographs were used for comparison. Transiliac bone biopsy was performed on 24 children with suspected primary osteoporosis based on frequent fractures and/or low BMD. Analysis of bone histomorphometry was performed using undecalcified samples. Histomorphometric findings were correlated with clinical data, and biochemical, radiographic, and densitometric findings.

In total 1396 fractures in 1373 children were recorded: the annual overall incidence of fractures in children under 16 years was 163/10 000. Boys sustained 63% of all fractures. Fracture incidence increased with age and was highest in puberty: in boys at 14 years (386/10 000) and in girls at 10 years (263/10 000). Forearm fracture was the most common fracture type, comprising 37% of all fractures. There had been an 18% decrease in the overall fracture incidence over the preceding 22 years, mainly due to the decrease in hand and foot fractures (-39% and -48%, respectively). The greatest decrease was seen for children aged 10 to 14 years (-30%). For the same period, a 31% increase of forearm fractures and 39% increase of upper arm fractures was observed. One fourth of the children with acute fracture reported a prior fracture, but only in 5% was the fracture history regarded as significant. These fracture-prone children (n=66) were found to have impaired bone health: on average, they had lower

calcium intake, physical activity level, and BMD than controls did. Vitamin D was below recommended level (50 nmol/L) in more than half of the patients and controls; low levels were associated with lower BMD in both groups. Asymptomatic vertebral compressions, and more hypercalciuria and hyperphosphaturia were also observed in the fracture-prone patients.

The poor resolution of low-radiation VFA compromised the accuracy in younger children and in those with low BMD. In older children close to their skeletal maturation and adult height, the visibility of VFA was mostly good.

Bone biopsy gives direct information on bone metabolism and is not influenced by subjects' skeletal size. It is an invasive method and this has limited the use in children. Bone biopsy findings in children with suspected osteoporosis were variable. Only 29% were found to have low trabecular bone volume in histomorphometric analysis. Bone turnover was low for age in one third and high in one third. Histomorphometric findings correlated poorly with DXA measurements or clinical data, underscoring the importance of this method in severe pediatric osteoporosis.

Lyhennelmä

Murtumat ovat yleisiä lapsuusiällä. Ilmaantuvuus vaihtelee maiden välillä ja on viitteitä lasten murtumien lisääntymisestä. Toistuvat murtumat, ja varsinkin nikamamurtumat, voivat olla merkki huonontuneesta luun laadusta. Kansainväliset ohjeistukset siitä milloin ja miten murtumalasten luustoa pitäisi tutkia, puuttuvat. Luuston mineraalitiheys on yksi luun lujuutta määrittävä tekijä ja sitä voidaan mitata luustontiheysmittauksella (DXA). Lapsen kasvava luusto vaikeuttaa kuitenkin näiden mittaustulosten, kuten muidenkin luun aineenvaihduntaa mittaavien parametrien tulkintaa. Tässä väitöskirjatyössä tarkasteltiin lasten murtumien ilmaantuvuutta ja erityispiirteitä. Tutkimme luustonterveyteen liittyviä asioita murtuma-alttiilla lapsilla. Arvioimme myös kahden harvemmin kliinisessä käytössä olevan menetelmän, VFA:n (DXA-menetelmään perustuva nikamamurtumien arviointi) ja luubiopsiasta tehdyn histomorfometrian, sopivuutta lapsipotilailla.

Kartoitimme lasten murtumien ilmaantuvuutta 12 kuukauden seurantajakson aikana vuonna 2005 väestöpohjaisessa aineistossa. Kaikki helsinkiläisten alle 16-vuotiaiden lasten murtumat rekisteröitiin terveyskeskus- ja sairaalapotilailla. Ne 4–16 -vuotiaat lapset, joilla todettiin merkittävä murtumahistoria, ohjattiin luustonterveystutkimukseen HYKS Lastenklinikalle. Merkittäviksi murtumiksi laskettiin kaikki vähäenergiaisista vammoista johtuneet nikamamurtumat, sekä toistuvat pitkien luiden murtumat (vähintään 2 murtumaa ennen 10 ikävuotta, tai vähintään 3 murtumaa ennen 16 ikävuotta). Tutkimuksissa mitattiin luuston mineraalitiheyttä, kalkki-aineenvaihduntaan liittyviä laboratorioparametreja, analysoitiin luuston röntgenkuvia ja elämäntapatekijöitä; ikä ja sukupuolivakioitua vertailuryhmää käytettiin murtumille altistavien riskitekijöiden kartoittamisessa. VFA-menetelmän osuvuutta testattiin 65 epäiltyä osteoporoosia sairastavan lapsipotilaan ryhmässä vertaamalla DXA-laitteen avulla saatuja selkärankakuvia perinteisiin selän röntgenkuviin. Epäiltyä vaikeaa primaaria osteoporoosia sairastaville lapsille tehtiin suoliluun harjanteen (transiliakaalinen) luubiopsia luuston tarkemman rakenteen selvittämiseksi histomorfometrialla. Näillä yhteensä 24 potilaalla oli ollut useita murtumia ja/tai poikkeavan matala luustontiheys DXA-mittauksessa. Histomorfometrialla saatuja tuloksia verrattiin kliinisiin ja ei-invasiivisten menetelmien tuloksiin.

Vuoden tutkimusjakson aikana alle 16-vuotiailla helsinkiläisillä todettiin yhteensä 1396 murtumaa 1373 lapsella: ilmaantuvuus oli 163 murtumaa 10 000 lasta kohden. Poikien osuus murtuma-lapsista oli 63 %. Murtumien määrä lisääntyi iän myötä ja ilmaantuvuus nousi korkeimmaksi murrosiässä, pojilla 14 vuoden (386/10 000) ja tytöillä 10 vuoden iässä (263/10 000). Yleisin lapsuusiän murtuma on kyynärvarren alueella, yhteensä 37 % kaikista murtumista. Tutkimusta edeltävän 22 vuoden aikana kaikkien murtumien ilmaantuvuus laski 18 %, johtuen suurimmaksi osaksi käsi- ja jalkaterämurtumien vähenemisestä (-39 % ja -48 %). Suurin lasku nähtiin 10–14 -vuotiailla (-30 %). Samana ajanjaksona kyynärvarren murtumien ilmaantuvuus on lisääntynyt 31 % ja olkavarren murtumien 39 %. Neljänneksellä murtumalapsista oli aikaisempia murtumia, mutta vain 5 %:lla murtumahistoria katsottiin merkittäväksi.

Jatkotutkimuksiin ohjautuneilla 66 lapsella oli keskimäärin selvästi heikentynyt luustonterveys: heillä oli matalampi luuston mineraalitiheys, ja vähäisempi kalkinsaanti sekä liikunta-aktiivisuus kuin vertailuryhmällä. Suurimmalla osalla potilaista ja vertailuryhmän lapsista mitattu veren D-vitamiinipitoisuus oli alle nykyisen suosituksen 50 nmol/L; matala D-vitamiinipitoisuus oli yhteydessä matalampaan luustontiheyteen sekä murtuman saaneilla että verrokeilla. Potilailla todettiin myös oireettomia nikamien kompressioita sekä verrokeita enemmän korkeita virtsan kalkki- ja fosfaattiarvoja.

VFA-menetelmän osuvuus lapsipotilailla nikamien kompressiomurtumien diagnosoinnissa jäi tavallisia röntgenkuvia heikommaksi huonon erottelukyvyn (resoluutio) vuoksi: varsinkin nuorilla potilailla ja niillä lapsilla, joiden BMD oli matala. Aikuispituuden jo lähes saavuttaneilla vanhemmilla lapsilla näkyvyys VFA-kuvissa oli hyvä.

Luubiopsia antaa tarkan kuvan luun aineenvaihdunnasta riippumatta potilaan koosta, mutta sen käyttöä lapsipotilailla on vähentänyt nukutuksen tarve toimenpiteen aikana. Tutkimuksessamme epäiltyä primaaria osteoporoosia sairastavien lasten histomorfometriset löydökset olivat vaihtelevia, matala hohkaluun määrä todettiin vain 29 %:lla. Luun aineenvaihdunta oli ikään nähden matalalla kolmanneksella, ja vastaavasti poikkeavan kiihtynyt kolmanneksella. Histomorfometriset tulokset olivat huonosti ennustettavissa muilla menetelmillä, mm. DXA-mittauksella. Tämä osoittaa luubiopsian tärkeyden yhtenä mahdollisena tutkimusmenetelmänä epäiltäessä primaaria osteoporoosia lapsilla ja toisaalta lasten luuston terveyden arvioinnin vaikeutta muilla mittareilla.

List of original publications

This thesis is based on the following publications:

I Mäyränpää MK, Mäkitie O, Kallio PE. Decreasing incidence and changing pattern of childhood fractures: A population-based study. *J Bone Miner Res* 2010 Dec;25(12):2752-9.

II Mäyränpää MK, Viljakainen HT, Toiviainen-Salo S, Kallio PE, Mäkitie O. Impaired bone health and asymptomatic vertebral fractures in fracture-prone children – A case-control study and guidelines for screening. *J Bone Miner Res* 2012 Feb 24. doi: 10.1002/jbmr.1579. (Epub ahead of print)

III Mäyränpää MK, Helenius I, Valta H, Mäyränpää MI, Toiviainen-Salo S, Mäkitie O. Bone densitometry in the diagnosis of vertebral fractures in children: accuracy of vertebral fracture assessment. *Bone* 2007 Sep;41(3):353-9.

IV Mäyränpää MK, Tamminen IS, Kröger H, Mäkitie O. Bone biopsy findings and correlation with clinical, radiological, and biochemical parameters in children with fractures. *J Bone Miner Res* 2011 Aug;26(8):1748-58.

The publications are referred to in the text by their roman numerals. These articles were reprinted with the kind permission of the copyright holders. In addition, some previously unpublished data are presented.

Abbreviations

25-OHD	25-hydroxyvitamin D, calcidiol
aBMD	areal bone mineral density
Ac.f	activation frequency
ALP	alkaline phosphatase
BFR/BS	bone formation rate per bone surface
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BV/TV	bone volume per tissue volume
Ca	calcium
COL1A1	collagen, type I, alfa 1
COL1A2	collagen, type I, alfa 2
Crea	creatinine
CT	computed tomography
D_3	vitamin D_3, cholecalciferol
DXA	dual-energy X-ray absorptiometry
ES/BS	eroded surface per bone surface
FGF-23	fibroblast growth factor 23
GH	growth hormone
ICTP	carboxyterminal telopeptide of type I collagen
IJO	idiopathic juvenile osteoporosis
ISCD	International Society for Clinical Densitometry
LBM	lean body mass
LRP5	low-density lipoprotein receptor-related protein 5
MAR	mineral apposition rate
Mlt	mineralization lag time
MRI	magnetic resonance imaging
MS/BS	mineralizing surface per bone surface
O.Th	osteoid thickness
Ob.S/BS	osteoblast surface per bone surface
Oc.S/BS	osteoclast surface per bone surface
OI	osteogenesis imperfecta
OS/BS	osteoid surface per bone surface
OV/BV	osteoid volume per bone volume
Pi	phosphate
PINP	procollagen type I N-terminal propeptide
pQCT	peripheral quantitative computed tomography
PTH	parathyroid hormone
SD	standard deviation
Tb.N	trabecular number
Tb.Sp	trabecular separation
Tb.Th	trabecular thickness
VFA	vertebral fracture assessment
W.Th	wall thickness
WHO	World Health Organization
Wnt	wingless-int

1 Introduction

Fractures are common in childhood. In order to make prevention programs for safer environments possible, the fracture epidemiology of specific geographical areas must be known. Finland is a Nordic country with sunny but quite short summers and snowy winters. Latitude and seasonal variation influence leisure-time activities and injury patterns.

Some propose that children break their bones because they are so small... active, or inactive, or don't eat properly... All these are every-day questions to physicians treating children with traumatic fractures, and have been so for a long time. We have rather accepted the fact that it is normal to break a bone as a child, even more so if the patient is a boy and his father did so too when he was young. Nevertheless, we do not know about all the risk factors for fractures in children, and some predisposing factors could probably be avoided.

The field of bone health assessment in children has changed during recent years due to the introduction of precise densitometers, new biomarkers, and advances in the field of genetic research. After the primary enthusiasm, all new methods have proven to have some limitations. Growing children are not easy to assess for bone health, as timing of growth and maturation varies, and the changing body size affects many parameters. Dual-energy X-ray absorptiometry (DXA) has become the gold standard in postmenopausal women to detect bone loss; it is the method of choice according to the WHO guidelines for diagnosing osteoporosis. In children, use of DXA has been widely studied, but remains challenging in clinical settings.

In this study, the aim is to thoroughly evaluate children who were suspected of having impaired bone health. An accurate new method for vertebral imaging with lower radiation dose than conventional X-ray was also of interest. The definite analysis of bone metabolism on the cellular level requires a bone specimen. Quantitative bone histomorphometry was described already in the 1960's by Harold Frost. The method is still rarely used in pediatrics because of its invasive nature, and especially due to the lack of normative data. This gap of missing reference values was filled in by the group of Francis Glorieux in 2000 (Glorieux et al. 2000). In the present study, iliac crest bone biopsies were performed in a group of children with fractures. Findings from both these rarely used methods in children were compared with results obtained by more conventional techniques.

2 Review of the literature

2.1 Bone

2.1.1 Bone structure and function

Bone and cartilage constitute the human skeleton, which has many functions: to support body weight, to provide mechanical support for posture and movements, to protect inner organs, and to serve as metabolically active storage for minerals such as calcium, phosphate, and magnesium (Seeman et al. 2006, Robey et al. 2008). The strength of bone is determined by its structural design and material composition. Bone must be stiff to resist deformation, flexible to absorb energy without cracking, and light to allow movements. Bones larger in diameter, with thicker walls, and with more bone material at a distance from the neutral axis, are stronger. There are subtypes of bone classified according to their shape: long bones (e.g. humerus, radius, femur, or tibia), short bones (cuboidal bones in foot and wrist), and flat bones (e.g., skull, clavicle, or ileum). Vertebrae are formed from the cylinder shape body (anterior part) and arch (posterior part). The function of the vertebrae, similar to that of long bones, is to support (body weight) and to protect (spinal structures).

Macroscopically, bone can be divided into cortical bone and cancellous (trabecular, spongy) bone. The long bones are tubular structures that contain a marrow cavity, so that the compact cortical mass is placed distant from the central long axis, conferring greater resistance to bending (Seeman et al. 2006). Cortical bone constitutes 80% of the bony skeleton and is mainly found around the shaft (diaphysis) of long bones. Trabecular bone represents 20% of the skeletal mass but 80% of bone surface. It is found mainly in the vertebral bodies, metaphyseal areas at the end of long bones, and in the flat bones. Trabecular bone is metabolically active and maintains the mineral homeostasis. The spaces between the trabecular meshwork are occupied by bone marrow.

Bone tissue is comprised of metabolically active cellular portion and matrix. Matrix is composed of minerals (50–70%), organic components (20–40%), and water (10%). Hydroxyapatite, a crystalline lattice compound of calcium, phosphate, and hydroxide, comprises 95% of the mineral component. As much as 99% of the body calcium is stored in the bone crystals. The organic component contains approximately 90% of collagen, and the rest is noncollagenous proteins, proteoglycans, and lipids. Type I collagen is the most abundant form of collagen in the body in connective tissue and bone (Dempster 2006).

2.1.2 Bone cells

Bone is a dynamic tissue, which remodels and repairs itself throughout life. The many diverse structural and metabolic functions of bone are principally driven by the interplay between just two cell types: osteoblast and osteoclast. Osteoblasts are responsible for the production of the bone matrix constituents and new bone. They originate from multipotent mesenchymal stem cells, which have the capacity to differentiate into osteoblasts, adipocytes (fat cells), chondrocytes (cartilage-forming cells), myoblasts (muscle cells) or fibroblasts (Hadjidakis et al. 2006). Osteoblasts initially produce osteoid by depositing collagen, and then initiate the mineralization by providing the enzymes required (e.g., alkaline phosphatase and osteocalcin). Up to 15% of the mature osteoblasts are entrapped in the new bone matrix and differentiate into osteocytes. Osteocytes are the most abundant (95%) cell type in bone matrix. They are found in lacunae and connected to each other and other cells with dendrites. Osteocytes are critical in the repair of micro-damages because of their ability to sense mechanical stress to bone (Bonewald 2011). Some of the osteoblasts remain on bone surface, becoming flat, inactive lining cells (Hadjidakis et al. 2006). Osteoclasts, originating from hematopoietic cells of the mononuclear lineage, are responsible for bone resorption. First, immature osteoclast precursors proliferate and fuse to form giant multinuclear cells. Mature resorptive cells attach to the calcified matrix, form a ruffled membrane against the bone surface, and resorb bone by acidification and proteolysis of the bone matrix (Teitelbaum 2000).

2.1.3 Bone growth and metabolism

The longitudinal growth of most bones occurs by endochondral ossification. At the ends of long-bones, cartilage tissue is first added to the growth zones (the growth plates) between epiphyses and metaphyses, and then the cartilaginous scaffold is transformed into bone tissue in the adjacent metaphysis with the help of osteoblasts and osteoclasts (Schoenau et al. 2003, Rauch 2005). Most of the tissue produced by the growth plate will eventually become diaphyseal bone; periosteal resorption occurs at the metaphyses by osteoclastic function (Baron 2003). Vertebral bodies are primarily cartilaginous, and form mainly from growth of the primary and secondary ossification centers (anular or ring epiphyses) on the superior and inferior edge of each typical vertebra. Vertebral bodies are mineralized bone at birth; epiphyses appear in radiographs during puberty around 12 to 15 years (Moore 1988).

The increase of bone mass from infancy to adulthood is almost 30 fold (Trotter et al. 1974). This is due to lengthening and widening of the bones, and accrual of higher bone mineral density (BMD). Gilsanz and co-workers measured by quantitative computed tomography (QCT) a 25% increase in lumbar spine volumetric BMD during puberty (Gilsanz et al. 1988). This effect is due to slow thickening of the trabeculae attributable to remodeling with a positive balance, not to number of trabeculae or change in material density, as demonstrated with bone biopsy by Parfitt et al. (Parfitt et al. 2000). Strength of tubular bones is gained by widening of the diameter and thickening of the cortex rather than increased volumetric density (Bornstein et al. 1987).

Modeling and remodeling

Modeling is bone's adaptation to mechanical forces; the shape, mass, and size change throughout life. In modeling, osteoclasts and osteoblasts are independently active on different surfaces of the bone (Parfitt et al. 2000, Rauch 2005) (Figure 1). Modeling usually results in a net increase in the amount of bone tissue, due to less active osteoclastic function in the inner (endocortical) surface, as compared to osteoblasts working on the periosteal surface without interruption. In addition to cortical thickening, modeling is also important for reshaping the long bones as they grow in length during childhood.

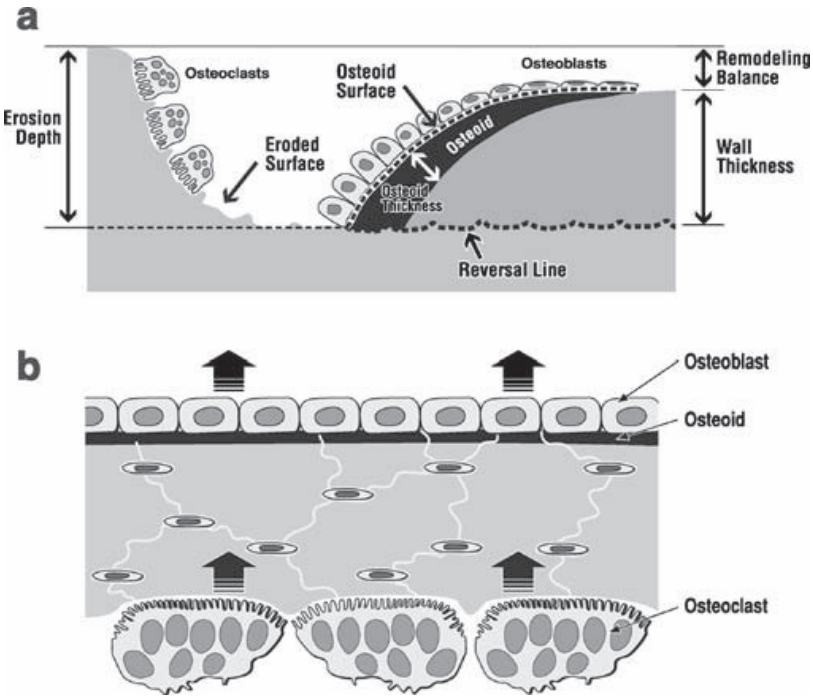


Figure 1. a) Schematic presentation of bone remodeling site in trabecular bone: renewing of bone matrix occurs in bone multicellular units, where the functions of bone resorption by osteoclasts followed by bone formation by osteoblasts are coupled. b) Bone modeling site. Osteoblasts and osteoclasts are located on opposite sides of a bone cortex and working independently (from Rauch 2006, reprinted with permission).

Remodeling is the process by which bone is continuously turned over by coordinated actions of resorption and formation. Remodeling allows the maintenance of the shape, quality, and size of the skeleton by repairing microfractures and by modifying the structure (Parfitt 2002). Remodeling occurs in bone multicellular units (BMU) as first

described by Frost in the 1960's (Frost 1966), formed by closely working (coupled) osteoclasts followed by the large group of osteoblasts. In normal mature bone, up to 80% of the cancellous bone surface and 95% of the intracortical surface is covered by lining cells. Thus, there is constant matrix remodeling of bone in up to 10% of all bone mass at any point in time; 25% of trabecular bone and 2–5% of cortical bone is replaced annually in adults (Baron 2003). The osteoclasts move in trabecular bone at a speed of approximately 25 μm/day, digging a trench with a depth of 40 to 60 μm. The remodeling cycle lasts about 3 to 4 months, where the phase of resorption is 2 to 3 weeks, reversal phase is 4 to 6 weeks, and the final formation phase, where osteoblasts lay down bone until the gap is completely replaced by new, is up to 4 months (Hadjidakis et al. 2006). During the growth period, about 5% additional bone is formed in every remodeling cycle as compared to resorption (Parfitt et al. 2000). The balance of the remodeling cycle in a young adult skeleton is close to zero, for as much bone is formed as is removed. After the fifth decade of life, bone formation rate fails to keep pace with resorption activity, and bone loss begins.

2.1.4 Regulators of bone

Local

There are local, systemic, and mechanic controls on the activity and growth of the bone. Functions of the two bone-specific cell types, osteoblasts/osteocytes and osteoclasts, are determined by secreted molecules that can be either cytokines acting locally, or hormones acting systemically. Bone cells themselves regulate the remodeling activity. Osteoblasts secrete alkaline phosphatase (ALP) and produce collagen to form osteoid. The bone isoform of ALP is an enzyme important for mineralization of osteoid; hypophosphatasia caused by genetic defects or other diseases leads to a large variety of skeletal problems with osteomalacia and fractures (Weiss et al. 1988). Marrow stromal cells, osteoblasts and osteocytes are all involved in the processes of osteoclast development, such as recruitment, differentiation, survival, fusion, and activation of osteoclasts. As the osteoblast transitions to an osteocyte, alkaline phosphatase secretion is reduced, and osteocalcin is elevated. Osteocalcin is a protein incorporated into the organic bone matrix; it is proposed to have some role also in glucose and insulin metabolism (Lee et al. 2007). Additional proteins secreted by the osteocytes include fibroblast growth factor 23 (FGF-23), involved in phosphate homeostasis, and sclerostin, a bone formation inhibitor by the wingless-int (Wnt) signaling pathway (Bonewald 2011). FGF-23 enhances phosphate excretion by the kidney, and elevated levels lead to reduced circulating phosphate levels and, consequently, osteomalacia and rickets (Fukumoto et al. 2007). Thus, although trapped inside the bone matrix, osteocyte is actively involved in the turnover of bone matrix through various mechano-sensory mechanisms and by regulating both osteoblasts and osteoclasts, and it plays a role in both phosphate metabolism and calcium availability.

Hormones

Several extra-skeletal hormones influence growth, such as growth hormone (GH), thyroxin, insulin, and corticosteroids (all of which influence growth rate), leptin (which alters body composition), parathyroid hormone (PTH) and vitamin D (these also affect skeletal mineralization and calcium homeostasis) (Table 1). The key hormone in growth is GH, which increases together with insulin-like growth factor 1 (IGF-1) in both sexes during puberty. The increase is most marked during puberty and correlates best with pubertal stage, bone age, and peak height velocity. The maturation of bones is influenced by thyroid hormones, adrenal androgens, and gonadal sex steroids, mainly estrogen. An excess secretion of these hormones can lead to advanced bone maturation, and deficiency causes a delay. During puberty, both sex steroids and GH participate in the pubertal growth spurt. Increased estrogen in girls leads to endocortical apposition, and in boys the rising levels of testosterone increase muscle mass and strength leading to increased bone cross-sectional size and cortical thickening (Schoenau et al. 2000, Schoenau et al. 2002b). The ending of the growth spurt is secondary to epiphyseal closure, due to the action of sex steroids. In males, the closure of growth plates occurs some years later than in females; the prolonged growth period leads to increased bone length and cortical thickness, but bone mineral content in trabecular compartment is similar. The stage of puberty is an independent determinant of BMD in girls, whereas weight is a more important determinant in boys (Boot et al. 1997).

Vitamin D and calcium

Vitamin D and calcium are essential for bone. In the skin, ultraviolet B radiation exposure induces the production of vitamin D_3 (cholecalciferol) from the 7-dehydrocholesterol. D_3 is then metabolized twice to become active: first in liver into 25-hydroxyvitamin D (25-OHD, calcidiol) and second, in the kidneys into 1,25-$(OH)_2$D (calcitriol). Vitamin D_3 can also be obtained from food: fatty fish and fortified dairy products (Holick 2007). Low vitamin D levels lead to impaired bone health by decreasing calcium absorption from the intestine, and decreasing the maximal reabsorption of phosphate. The best sources of calcium are various dairy products, but also green leafy vegetables and fish. Low levels of calcium in the blood induce PTH secretion from the parathyroid gland; there is also a negative correlation between serum concentrations of 25-OHD and PTH. A threshold level of serum 25-OHD, above which serum PTH plateaus, is between 50 and 75 nmol/L (Dawson-Hughes et al. 2005). PTH is needed to mobilize calcium from the bone to correct hypocalcaemia. Long-lasting increase in PTH secretion leads to bone loss, but intermittent high PTH is anabolic for bone. There are PTH receptors on the osteoblasts, and their activation by hormone prolongs osteoblast life and increases activity (Potts 2005). Vitamin D is also necessary for skeletal growth during infancy and early childhood. Low maternal vitamin D status affects bone growth in early infancy (Viljakainen et al. 2011a), and is associated with lower BMC in children at 9 years of age (Javaid et al. 2006).

Table 1. Most important hormones affecting bone growth and turnover.

Name	Mainly from	Effect on bone
Growth hormone (GH)	Pituitary gland	Increases the rate of mitosis in chondrocytes and osteoblasts, increases the rate of protein synthesis (collagen, cartilage matrix, and enzymes for cartilage and bone formation)
Insulin-like growth factor 1 (IGF-1)	Liver	Stimulates cell growth and proliferation at the growth plates and bone; mediator of GH actions
Parathyroid hormone (PTH)	Parathyroid gland	Increases the resorption of calcium from bones to the blood, thereby raising blood calcium levels and increases the absorption of calcium in the small intestine and kidneys; increases the number and activity of osteoblasts
Calcitonin	Thyroid gland	Decreases the resorption of calcium from bones, thereby lowering blood calcium levels; inhibits the activity of osteoclasts
Estrogen	Ovaries	Induces maturation of the skeleton; increases bone mineral apposition
Testosterone	Testes	Increases bone mineral apposition; increases muscle mass
Leptin	Adipocytes	Regulates the balance between osteoblasts and osteoclasts
Vitamin D	Food/skin – liver – kidneys	Increases calcium absorption from intestine and phosphate reabsorption in the kidneys
Thyroxine	Thyroid gland	Increases the rate of protein synthesis and increases energy production from all nutrients
Fibroblast growth factor 23 (FGF-23)	Osteoblasts	Reduces the reabsorption of phosphate in the kidneys
Osteocalcin	Osteoblasts	Regulates bone mineralization and turnover; stimulates pancreas to release insulin and the testes to release testosterone

A longitudinal study by Bailey et al. with BMC measurements of the whole body, estimated that 26% of adult calcium mass is laid down during the two adolescent years of peak skeletal growth, the amount estimated equivalent to that lost later in life (Bailey et al. 2000, Cooper et al. 2006). This also explains the high need for daily calcium intake during these years. According to national nutrition recommendations in Finland, children up to 6 years should get 600 mg per day, children from 6 to 9 years 700 mg per day, and adolescents from 10 to 18 years 900 mg of calcium per day. Due to the northern location of the country, a national recommendation for daily vitamin D supplementation is also given: 10 µg (400 IU) for children under 2 years of age and

7.5 µg (300 IU) for all children of 2 to 18 years (the National Nutrition Council 2011). Before the year 2011, recommendation for intake of vitamin D was 7.5 µg for all children; thought to be obtained from healthy food. Supplementation with vitamin D was only adviced for children under 3 years (10 µg).

Low calcium and phosphate result in decreased bone mineralization; failure or delay in calcification of the osteoid leads to osteomalacia in adults. In growing bones, hypophosphatasia also impairs the expected apoptosis of hypertrophied chondrocytes, with cellular "ballooning" and disorganization of the growth plate. Demineralized collagen matrix is prone to dehydration and swelling, causing bone pain by expanding the periosteum outwards. Osteomalacia in immature bones is referred to as rickets. The term rickets also describes the abnormal organization of the cartilaginous growth plate and the accompanying impairment of cartilage mineralization. Clinically, rickets usually presents with bone pain and muscle weakness; radiographic features include thinning of cortex in long bones, and widening of the growth plates and metaphyseal areas in tubular bones. Delayed fusion of the skull fontanels may be seen in infants. Rickets can also be associated with poor growth, abnormal teeth enamel, and susceptibility to infections. The most common form of rickets is associated with vitamin D deficiency (Misra et al. 2008).

Mechanical loads

Hormones and nutrition play a modulating role, but bone development is predominantly controlled by local factors in response to the mechanical stimuli that act on the bone. It was proposed, as early as the 1870's by orthopedic surgeon Julius Wolff, that altered mechanical usage can cause a bone to change its architecture (Wolff 1870). The strength of bones is dependent on the quality and size. While the potential size of bone and muscles are determined mainly by genetic factors, the actual development of muscle and bone during growth is influenced by forces associated with gravity (body mass) and physical activity (Schoenau et al. 2002a). Bone size and mineral density are influenced by physical stimuli (strain); low usage swifts bone to a state of low remodeling, resulting in thinner cortices. Higher strain, up to a certain point, accelerates bone formation and induces bone modeling, leading to thicker trabeculae and cortices. At some point, strain overpasses the bone's ability to adapt, and fracture occurs. The intrinsic control of bone whether new bone is added or taken away from the skeleton is called "mechanostat", a theory described by Frost, and a refinement of the Wolff's law (Frost 1987). The mechanostat theory has been updated with results from new methods assessing bone, and the sensing role of osteocyte is thought to be crucial (Frost 2003, Hughes et al. 2010). Already in 1961, Maresh published data obtained from radiographs of children during their first six years of life, and showed similar periosteal apposition rates in the femur and humerus at age 12 months, but four times faster in the femur at age 33 months (Maresh 1961). Disorders that result in absence or removal of mechanical stimuli during growth, such as cerebral palsy, spina bifida, or poliomyelitis, lead to thin bones in the affected extremities (Gooding et al. 1965, Roh et al. 1973, Ráliš et al. 1976). On the other hand, due to altered mechanical stimuli, rapid periosteal apposition can occur as well. In experimental and patient studies with large bony defects, the neighboring bones

strengthen and replace the weight-bearing function of the removed bone (Goodship et al. 1979, Falder et al. 2003).

Heritability

Height and other anthropometric variables related to skeletal size have long been known to be highly heritable (Clark 1956). Several genomic regions have been linked to cross-sectional bone size and bone width, but no specific genes have yet been singled out as major contributors (Rauch 2005). Bone mass and peak bone mass are highly regulated by hereditary factors (Smith et al. 1973, Dequeker et al. 1987, Peacock et al. 2002). It is estimated that as much as 80% of bone mass is determined by genes and only 20% can be explained by environmental factors (Howard et al. 1998). The heritability of fracture tendency, as expected with such a complex phenotype, is not strong even in large twin studies (Kannus et al. 1999b, Peacock et al. 2002). Specific bone disorders leading to decreased bone mass and fragility fractures are rare, although several hundred forms have been described in the literature. Genetic defects affecting collagens or proteins involved in the Wnt signaling pathway have been identified. For example, mutations in *COL1A1* or *COL1A2* genes are responsible for the reduced expression of type I collagen, causing fragile bones in the majority of the patients with osteogenesis imperfecta (OI) (Rauch et al. 2004). More than 2000 different collagen type I mutations have been identified in OI patients to date (Dalgleish 1997), and listed in a database (www.le.ac.uk/ge/collagen/). LRP5 is a cell transmembrane protein, acting as a receptor in the Wnt signaling pathway. The gene encoding the low-density lipoprotein receptor–related protein 5 (LRP5) has been shown to be associated with bone mass accrual during growth, as well as BMD and fractures in both children and adults (Gong et al. 2001, Little et al. 2002, Hartikka et al. 2005, van Meurs et al. 2006, Saarinen et al. 2007, Saarinen et al. 2010). Variable phenotypes can be caused by mutations in one gene; on the other hand, mutations in different genes can result in similar phenotypes (Michou et al. 2011, Mäkitie 2011). Some of the most common genetic conditions with bone fragility are described in more detail in Section 2.3.3.

2.2 Bone health assessment

2.2.1 Growth and maturation

Normal growth in childhood not only includes growth of the skeleton in size, but also sexual maturation, body shape changes, weight gain, and increased height velocity in adolescence. At puberty, both sex steroids and GH participate in the pubertal growth spurt. The ending of the growth spurt is secondary to epiphyseal closure, due to the action of the sex steroids. The skeletal maturation or bone age of a child can be determined by assessing appearance of growth centers — for example by radiographs of the hand and elbow as described by Greulich and Pyle (Greulich et al. 1959). Assessment of the pubertal maturation is based on physical signs: breast development in females, genital development in males, and pubic hair development in

both males and females (Tanner 1962). There is a 4- to 5-year variance in the onset of puberty, and the timing is largely genetically regulated (Palmert et al. 2001). In females, the beginning of puberty is around 10 years and the peak height velocity occurs 1 to 2 years later (mean 11.5 years). In males, the sexual development starts usually around age 11 years, and the timing of the rapid growth period is 2 to 3 years later (mean 13.5 years) (Neinstein et al. 2002).

2.2.2 Biochemical methods

Bone consistently undergoes remodeling to renew its damaged microstructure. Bone markers are specific bone-derived molecules that can be classified into bone formation and bone resorption markers and are present in serum or urine. The bone formation markers, alkaline phosphatase (ALP) and osteocalcin, reflect the activity of osteoblasts, and procollagen type I N-terminal propeptide (PINP) also the synthesis of collagen type I (Yang et al. 2006). The bone resorption markers are released upon bone matrix degradation mediated by osteoclasts. They include the short N- and C-terminal fragments of collagen molecules: the C-terminal telopeptide of type I collagen (ICTP) measured from serum and NTx or CTx measured in urine. All biochemical bone markers are dependent on bone mass and number of bone cells, as well as on remodeling activity. In adults, bone markers are considered to reflect remodeling activity, or the metabolic activity of the bone, but they do not provide information about remodeling balance. In children, modeling and endochondral bone formation related to growth contribute to the total bone turned over but with an unknown extent at a given time. This makes biochemical assessment of bone turnover difficult in children (Schoenau et al. 2003, Huang et al. 2011). The bone markers in pediatrics are more valuable in the longitudinal follow-up or during treatment.

Despite marked variations in intake, the circulating concentrations of the main skeletal minerals, calcium and phosphate, are strictly regulated by endocrine mechanisms that show little variation with age (Schoenau et al. 2003). Therefore, calcium or phosphate levels in the blood or urine are not reflective of skeletal reserves, but may reveal an underlying condition such as metabolic bone disease, kidney disease, or parathyroid dysfunction. The best available indicator for total body vitamin D status is the circulating serum 25-OHD (Misra et al. 2008). The biologically active metabolite 1,25-$(OH)_2D$ is only measured in disorders of mineral metabolism and kidney dysfunction and has little value in the evaluation of vitamin D status due to its instability and extremely small concentration.

2.2.3 Radiological methods

Radiography

Conventional radiographs remain the gold standard in the evaluation of bone. Almost all fractures can be detected from radiographs. They are also used to determine the bone age and in primary assessment of focal abnormalities. In addition, skeletal features of bone dysplasias can be assessed, as well as ricketic abnormalities detected and scored from long-bone X-ray images (Thacher et al. 2000). When evaluating the spine after injury, plain radiographs are valuable; computed tomography (CT) and magnetic resonance imaging (MRI) are sometimes needed to clarify any suspected fractures and in evaluating the soft-tissue structures. There are several methods of classifying thoracolumbar fractures (Newton et al. 2010). The Denis classification includes compression, burst, flexion-distraction, and fracture-dislocations of the vertebral body and posterior structures (Denis 1983), and is proposed to be valid in most traumatic adolescent spinal injuries. In younger children, vertebral trauma may involve the apophysis (end plates) partly or not at all radiographically visible, complicating the diagnosis (Clark et al. 2001).

When looking for signs of osteoporosis, that is vertebral compression fractures in the spine, plain radiographs are used. Vertebral body morphology can be assessed from a lateral view and this is used for classifications of compression fractures. Anterio-posterior view is also used to detect compressions, developmental abnormalities, or scoliosis. Normal variations in healthy children include minor wedging of the vertebral body, anterior aspect in the thoracic spine, and posterior aspect in the lumbar spine, as well as rounded anterior margins due to non-visible ring-apophysis in young children. Vertebral morphology in pediatric population has been characterized in a study by Mäkitie et al. (Mäkitie et al. 2005). Deformities in children with secondary osteoporosis were classified by the anterior wedging or middle compression of vertebral body; height loss of more than 20% as compared to adjacent vertebrae was determined abnormal. Grade 2 characterizes the progressive stages of anterior wedge deformity (2a, mild 20−49%, or 2b, severe > 50%), and Grade 3 the progressive stages of middle compression deformity (3a, mild < 30%, or 3b, severe > 30%). The semi-quantitative technique developed by Genant et al. (Genant et al. 1993) is widely used with adults to assess osteoporotic vertebral fractures. Genant's classification grades severity of vertebral compression as mildly deformed (Grade 1, 20–25% reduction of anterior, middle, and/or posterior vertebral body height), moderately deformed (Grade 2, 26–40% reduction in any height), or severely deformed (Grade 3, > 40% reduction in any height) (Figure 2).

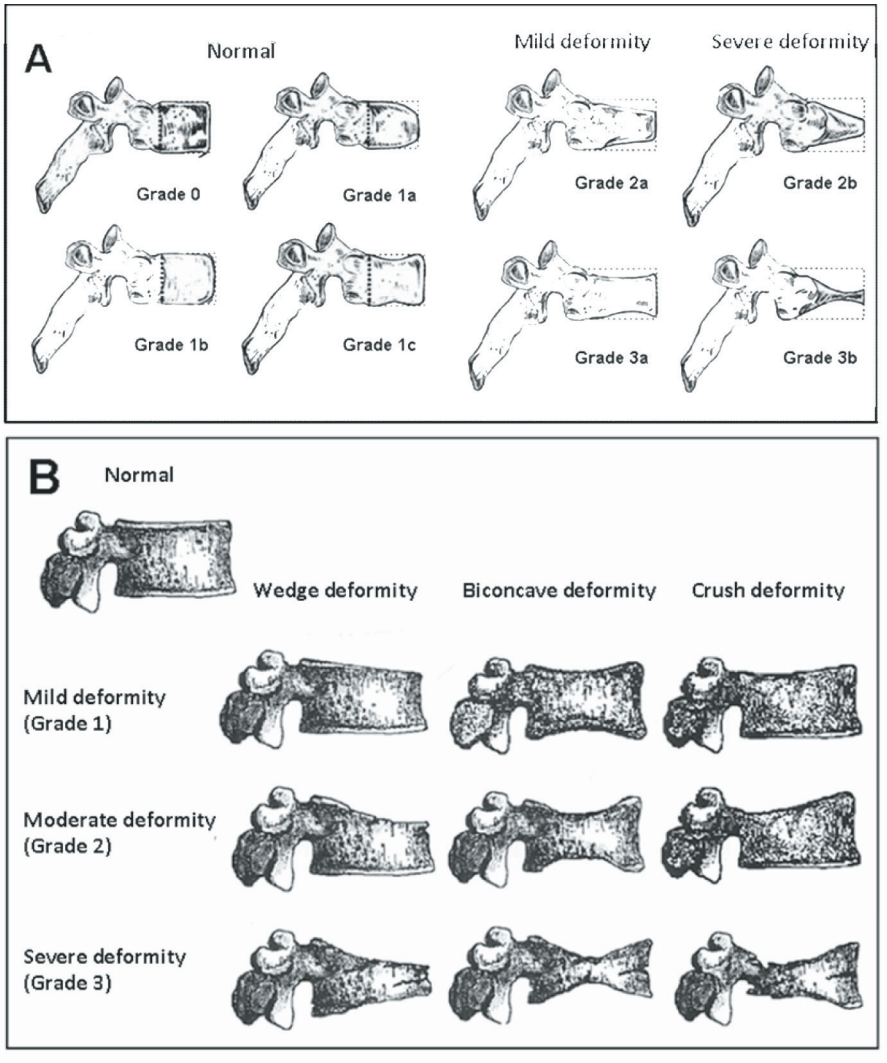


Figure 2. Illustrations of two vertebral morphology classifications: A) changes in children by Mäkitie et al. Grades 0 and 1 represent variants of normal; Grades 2a and 2b, progressive stages of anterior wedge deformity; and Grades 3a and 3b, progressive stages of compression deformity. B) Genant's classification of vertebral compression fractures in adults (adapted from Mäkitie et al. 2005 and Genant et al. 1993, reprinted with permission).

Vertebral fracture assessment

Vertebral fracture assessment (VFA) is a radiographic method using DXA device (densitometer) to assess vertebral body deformities during bone density measurement (Figure 3). VFA, also called morphometric X-ray absorptiometry (MXA), is based on the six-point measures of each vertebral body at 4th thoracic (Th4) to 5th lumbar (L5) from lateral view (Steiger et al. 1994, Damiano et al. 2006). The single energy fan beam is used, and in contrast to conventional cone beam radiography, the beam remains parallel to the vertebral endplates. This allows a better definition of the vertebral dimensions for a morphometric analysis, even though resolution and signal to noise ratio are both worse than in X-ray due to significantly lower radiation dose (about 1% of radiographs for whole spine). VFA is used in adults for identifying vertebral fractures in patients at risk for osteoporotic changes, and Genant's visual semi-quantitative method (Genant et al. 1993) is recommended for diagnosing vertebral fracture with VFA (Lewiecki et al. 2008). No data on the accuracy of VFA in pediatric patients has been available.

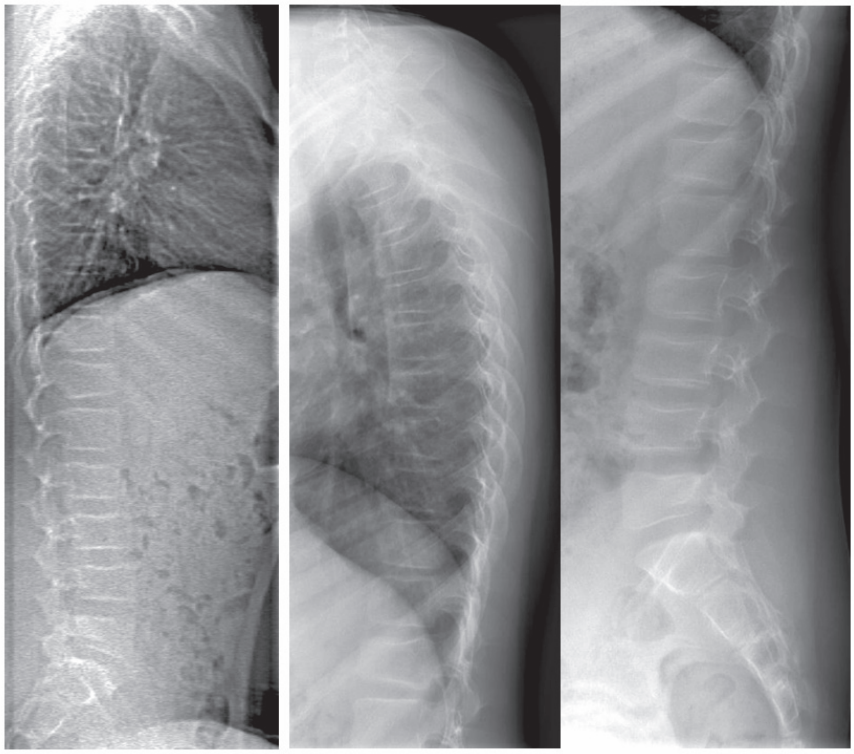


Figure 3. Spinal images of a 10-year-old child with several vertebral compressions. On the left, a whole spine vertebral fracture assessment (VFA) image obtained with densitometric imaging (Discovery A, Hologic). Standard radiographs of thoracic spine (middle) and lumbar spine (right) of the same patient.

Densitometry

Mineral density of bone is a radiographic measure of the amount of bone material as measured by absorption. Single photon absorptiometry was introduced in the 1960's, enabling the non-invasive quantitative assessment of bone mineral content (BMC) at peripheral sites of the skeleton: calcaneus and ultradistal radius (Cameron et al. 1963). Replacement of the radionuclide source with X-ray resulted in better precision and spatial resolution. Single energy measurements, however, are not possible at sites with variable soft tissue thickness and composition. Therefore, dual-energy X-ray absorptiometry (DXA) was developed and introduced in the 1980's, making the assessment of the axial skeleton, hip, or whole body possible. In addition, DXA achieved better resolution and a scanning time of the whole human body in less than one minute (Mazess et al. 1989, Genant et al. 1996). DXA has become a standard method for assessing bone density by noninvasive means, and it is a valid method to diagnose osteoporosis and to predict the risk of fracture in adults (Cummings et al. 2002). The most common sites measured with DXA are lumbar spine, hip (total hip or proximal femur), and whole body. In adults, the femur is of special clinical interest for assessment for risk of fractures. Lumbar spine measures are considered to reflect bone health of the trabecular bone, and the whole body DXA values reflect bone health of the long bones. Separate measurements can be done of any bone; special interest has been also on distal radius due to the high fracture incidence at this site.

DXA provides a calculated areal BMD (aBMD) by measuring bone mineral content (BMC) and bone area. Due to the planar, two-dimensional nature of the measurement technique DXA cannot determine true volumetric BMD. Several mathematical formulas to calculate three-dimensional densities have been developed, for correcting the effect of size and the known cylinder shape of human vertebrae (Carter et al. 1992, Kröger et al. 1992). For clinical practice, the projected areal density and estimation of cubic shape is used and aBMD results are transformed into age- and sex-specific Z-scores. T-score, used in adults, is the standard deviation of BMD from a healthy 30 year old of the same sex and ethnicity, representing the peak bone mass.

DXA in children is well tolerated, as it is rapid and non-invasive, and the radiation exposure is low. There are certain challenges in using DXA in growing children: technical issues with acquisition of the data from small bones with low mineral content, and with interpretation of the data (Gordon et al. 2008). To avoid an overestimation of bone mineral deficits in children, the BMD scores are compared to reference data for the same sex and age (Z-score), not to the mean of young adults (T-score) with peak bone mass (Figure 4). If the bone age, commonly determined with X-ray of the hand, is delayed or advanced, the BMD can be adjusted to the bone age instead of calendar age. Height adjustments are recommended in growth disturbances, and the whole body BMD is proposed to be assessed relative to height in children (Leonard et al. 2004). There is substantial variation in the normative data published by various manufacturers of DXA equipment, and for different scanners from the same producers; therefore, only reference data provided for the machine used are recommended for Z-score calculations in children (Genant 1995).

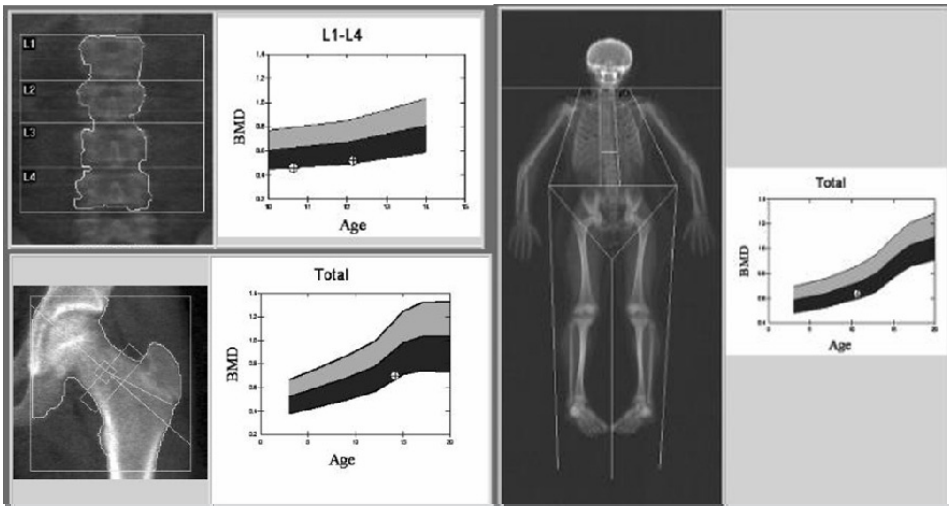


Figure 4. Assessment of bone mineral density (BMD) by dual-energy X-ray absorptiometry, DXA (Discovery A, Hologic). Areal BMD results are transformed into Z-scores and presented with age- and sex-specific reference curves. Measured sites lumbar spine, total hip, and whole body.

Other non-invasive techniques for measuring bone density include quantitative ultrasound (QUS), quantitative CT (QCT), peripheral QCT (pQCT), and quantitative magnetic resonance (QMR) at different body sites. In addition, radiographic absorptiometry and morphometry of the metacarpals have been used. The lack of sufficient evidence limits their use in clinical practice; only peripheral measurements at heel (with ultrasound) or distal radius (with pQCT) are validated for fracture prediction in postmenopausal women (Lewiecki et al. 2008). Although all methods report BMD, measurements from different devices cannot be directly compared (Genant et al. 1996). In children, reference data are not sufficient for the clinical use of QUS or pQCT for fracture prediction or diagnosis of low bone mass (Zemel et al. 2008).

2.2.4 Bone biopsy and histomorphometry

Bone histomorphometry of an undecalcified tissue sample is a method to directly obtain quantitative information on bone. It has been a key tool in assessing bone metabolism and structure, since Harold Frost first pioneered the technique in the 1960s (Frost 1966). Together with tetracycline labeling prior to sample collection, it offers a possibility to study bone cell function *in vivo*, as well as qualitative histologic assessment of bone structure. Once obtained, biopsy sample may also be assessed for direct three-dimensional bone architecture and mineral density by micro-CT. Histomorphometry can be performed in any bone, but the anterior aspect of iliac bone has proven to be a convenient site: easily feasible for the operator and for the patient,

and no side effects other than transient local discomfort or pain (Recker 2008). Iliac bone is the site commonly used to aspire bone marrow for examination (posterior iliac spine), and for obtaining pieces of bone for bone grafts (anterior iliac spine). In children, the normative histomorphometric data is available only for the iliac bone, published by Glorieux and co-workers (Glorieux et al. 2000).

Bone biopsy technique

Iliac bone biopsy can be taken using either horizontal or vertical technique. In horizontal approach, the bone sample is obtained from the iliac bone at a standardized site 2 cm posterior and 2 cm inferior to the anterior superior iliac spine (Figure 5) (Bordier et al. 1964, Hodgson et al. 1986, Recker et al. 2002). From a small skin opening, the bony surface is approached by blunt dissection through the gluteal muscles. A manual trochar or electric drill can be used to advance through the iliac bone. The core diameter of the trochar should be at least 5 mm (or preferably, 7 mm) to avoid fractured or crushed samples. The biopsy set consists of four parts: a pointed trochar; guide sleeve with sharp, serrated edges; trephine biopsy needle; and blunt extractor. For quantitative histomorphometric analysis, it is essential to have a full depth sample with two cortices separated by trabecular compartment, and of good quality without fractures or crushing. A good sample requires a horizontal approach, and in children, the biopsy should be done well below the iliac crest growth plate, to avoid growth cartilage (Rauch 2003). Local anesthesia is applied on both sides of the iliac bone, and in children, usually a general anesthesia is needed.

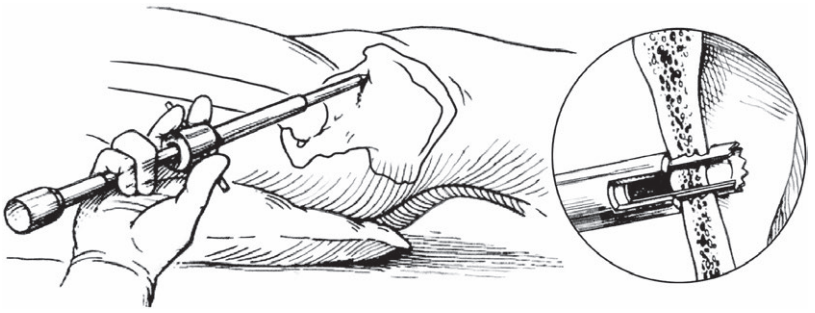


Figure 5. Schematic presentation of transiliac bone biopsy. Biopsy site is identified at the lateral side of the anterior iliac bone. A horizontal full depth bone sample with two cortices and a trabecular bone compartment in between is obtained by drill (adapted from Hodgson et al. 1986, reprinted with permission).

Histomorphometric analysis

For routine histomorphometry, the bone specimen is placed in 70–100% ethanol or 10% buffered formalin at room temperature for fixation for at least 48 hours. Then, the specimen is dehydrated in absolute ethanol and embedded in methyl metacrylate. Undecalcified sections of 3 µm are cut with microtome for staining (Figure 6). Unstained sections of 6 to 10 µm are used for polarized light and fluorescence microscopy. The actual histomorphometric analysis requires a high-quality microscope and a trained person. Even though today the manual or point-counting measurements are replaced with computerized systems allowing automated analysis, the judgment to identify correctly the histoanatomical components relies on the experience and subjectivity of the human operator (Parfitt 1993).

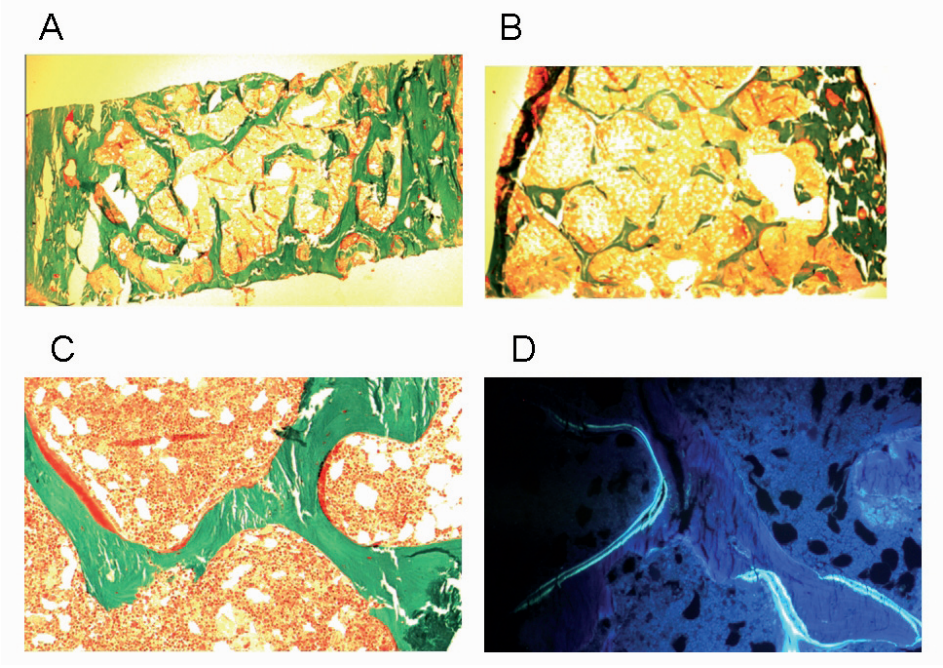


Figure 6. Histology of bone biopsy sample. A) Section of transiliac biopsy specimen with cortices in both ends and trabecular compartment in the middle. Normal bone volume. Original magnification 20x. Masson-Goldner trichrome stain: mineralized bone stained in green, bone marrow and cells in red. B) Bone sample from a child with low bone volume. Magnification 20x. C) Trabecular bone. Bone trabeculae in green, osteoid seams in red. Magnification 200x. D) Tetracycline double label in trabecular bone, visible in fluorescent light. Magnification 200x.

Introduction of standardized nomenclature, symbols, and units in 1987 facilitated the reporting of histomorphometric results (Parfitt et al. 1987). The parameters are classified into four categories: structural parameters, static bone formation parameters, dynamic formation parameters, and static bone resorption parameters (Table 2). Structural parameters are descriptives for bone sample, bone cortex, and trabecular bone structures. Although the histomorphometric measurements of lengths, areas, or cell counts are performed in two-dimensional sections, some ratios are converted to volumes and expressed with three-dimensional terminology to highlight the three-dimensional nature of bone. Static bone formation parameters include measurements of the unmineralized osteoid seam relative to the amount of mineralized bone. Dynamic formation parameters yield information on *in vivo* bone cell function, and can only be determined when fluorochrome (such as tetracycline) labeling is performed prior to obtaining the biopsy. Tetracycline incorporates into the bone at the front of the calcification. Mineralizing surface per bone surface (MS/BS) represents the percentage of bone surface exhibiting mineralizing activity. Mineral apposition rate (MAR) is the distance between two labels divided by the time between the midpoints of the labeling interval, and it reflects the activity of individual teams of osteoblasts. Bone resorption is measured with the number of osteoclasts per bone perimeter, or more preferably, by the percentage of bone surface that is in contact with osteoclasts (Oc.S/BS). The percentage of bone surface presenting a scalloped or ragged appearance of the bone–bone marrow interface, eroded surface (ES/BS), is also a measure of resorption (Rauch 2003).

Clinical applications

Histomorphometry has been used in humans to study the cellular basis of age-related bone loss in osteoporosis and the effects of pharmaceutical therapies (Han et al. 1997, Boivin et al. 2000, Seeman et al. 2006, Bala et al. 2011). Further, bone biopsies have been valuable in understanding the remodeling defect in renal bone disease and other metabolic diseases (Parfitt 2003). In children, bone biopsy has been helpful in characterizing the histological features of the two most common types of primary osteoporosis, OI, (Ste-Marie et al. 1984, Rauch et al. 2000b) and idiopathic juvenile osteoporosis (Jowsey et al. 1972, Rauch et al. 2000a, Rauch et al. 2002a). Furthermore, histomorphometry may be helpful in elucidating the underlying cause of osteoporosis and differentiating primary osteoporosis from osteomalacia. In Shwachmann-Diamond syndrome, a rare genetic disorder, the skeletal features were long thought to be due to malnutrition and pancreatic insufficiency, but the analysis of bone biopsies showed primary low-turnover osteoporosis (Toiviainen-Salo et al. 2007). A limited number of studies are available on histomorphometry in many conditions causing secondary osteoporosis in children. Renal bone disease and inflammatory bowel diseases result in abnormalities in bone mineral metabolism and in growth, which both make the use of DXA challenging (Hodson et al. 1982, Sanchez et al. 1998, Sanchez 2008, Ward et al. 2010). In such situations, bone histomorphometry may give more precise information about bone metabolism and quality.

Table 2. Commonly used histomorphometric parameters in children (modified from Rauch 2006, reprinted with permission).

Parameter	Abbr.	Significance
Structural Parameters		
Core width (mm)	C.Wi	Overall size of the biopsy specimen
Cortical width (mm)	Ct.Wi	Distance between periosteal and endocortical surfaces
Bone Volume / Tissue Volume (%)	BV/TV	Space taken up by mineralized and unmineralized bone relative to the total size of a bone compartment
Trabecular thickness (µm)	Tb.Th	Trabecular thickness
Trabecular Number (/mm)	Tb.N	Number of trabeculae that a line through a trabecular compartment would hit per millimeter of its length
Trabecular Separation (µm)	Tb.Sp	Mean distance between two trabeculae
Static Formation Parameters		
Osteoid Thickness (µm)	O.Th	Distance between the surface of the osteoid seam and mineralized bone
Osteoid Surface / Bone Surface (%)	OS/BS	Percentage of bone surface covered by osteoid
Osteoid Volume / Bone Volume (%)	OV/BV	Percentage of bone volume consisting of unmineralized osteoid
Osteoblast Surface / Bone Surface (%)	Ob.S/BS	Percentage of bone surface covered by osteoblasts
Osteoblast Surface / Osteoid Surface (%)	Ob.S/OS	Percentage of osteoid surface covered by osteoblasts
Wall Thickness (µm)	W.Th	Mean thickness of bone tissue that has been deposited at a remodeling site
Dynamic Formation Parameters		
Mineralizing Surface / Bone Surface (%)	MS/BS	Percentage of bone surface showing mineralizing activity
Mineral Apposition Rate (µm/d)	MAR	Distance between two tetracycline labels divided by the length of the labeling interval
Mineralization lag time (d)	Mlt	Time interval between the deposition and mineralization of matrix
Bone Formation Rate / Bone Surface ($\mu m^3/\mu m^2/y$)	BFR/BS	Amount of bone formed per year on a given bone surface
Static Resorption Parameters		
Eroded Surface / Bone Surface (%)	ES/BS	Percentage of bone surface presenting a scalloped appearance
Osteoclast Surface / Bone Surface (%)	Oc.S/BS	Percentage of bone surface covered by osteoclasts

2.3 Osteoporosis

2.3.1 Definition

Osteoporosis (“porous bones", from Greek: ὀστέον/osteon meaning "bone" and πόρος/poros meaning "pore") is a skeletal disease characterized by decreased bone mass and impairment of micro-architecture and strength of bone tissue, leading to increased bone fragility and fractures. This definition was provided by a WHO working group (Consensus development conference 1993). During the last decades, osteoporosis has become a serious worldwide public health issue in ageing populations (Sambrook et al. 2006). The underlying mechanism in all cases of adult osteoporosis is an imbalance between bone resorption and bone formation. The most common state, postmenopausal or type I osteoporosis, is due to estrogen loss in middle-aged women. Type II osteoporosis is related to age and occurs later in life; globally, it is of increasing importance due to longer life expectancy. In adults, the diagnosis of osteoporosis is based on low bone mineral content (BMC) or density (BMD) assessed by DXA. The measured BMD in post-menopausal females is transformed into T-scores by comparing with the average BMD of young adults of the same sex at the time of peak bone mass; the difference is presented in standard deviation (SD) units and used to define osteoporosis. A T-score below -2.5 is considered as osteoporotic, and values from -1.0 to -2.5 as “low bone mass” or “low bone density” (Lewiecki et al. 2008). Women with BMD or BMC T-score values of -2.5 or below, in the presence of at least one fragility fracture, are considered as having severe osteoporosis. Typical osteoporotic fractures in the elderly are those of vertebrae, hip, and distal radius (Rachner et al. 2011). In pre-menopausal females, and in men under age 50, a sex- and age-adjusted Z-score, not T-score, is preferred. A Z-score of -2.0 or lower is defined as “below the expected range for age”, and a Z-score above -2.0 is “within the expected range for age”.

Indications for BMD testing with DXA in adults is suggested to include all women aged 65 and older, and all men aged 70 and older (Lewiecki et al. 2008). All younger adults with risk factors for fracture (that is: a prior fracture, underlying disease, or condition associated with low bone mass or bone loss), as well as those with medications or being considered for therapy associated with bone effect, are recommended to be screened with DXA. In post-menopausal women, a decrease of one standard deviation of BMC or BMD predicts two to three times increase in the risk of future fracture (Gärdsell et al. 1991). Age and life-style factors have also been reported to be an independent risk factor for fractures, regardless of the BMD level (Nguyen et al. 2007). The prediction of hip fractures and other osteoporotic fractures can be made based on the validated assessment algorithms, which include clinical risk factors alone, or the combination of clinical risk factors and BMD. These algorithm tools (available at www.shef.ac.uk/FRAX or www.fracturerisk calculator.com) are suitable for men and women, and results can be used to economic optimization of population level screening and for individual treatment planning.

2.3.2 Osteoporosis in children

For long, there had been no consensus on the diagnostic criteria for pediatric osteoporosis. According to the first Pediatric Official Positions of the International Society for Clinical Densitometry (ISCD) given in 2007, 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 (Rauch et al. 2008). A clinically significant fracture history was proposed to include at least one of the following: i) a vertebral compression fracture, ii) one lower extremity long bone fracture, or iii) two or more upper extremity long bone fractures. Low BMC or areal BMD is defined as a Z-score of less than or equal to -2.0, adjusted for age, sex, and/or body size, as appropriate. The term "low BMC/BMD for chronological age" is to be used if the child's Z-score is below -2.0, stature is normal for age, and no fracture history is present. Recommended sites for DXA measurement in growing children are the lumbar spine and total body, as hip (total hip or femur) is vulnerable to the significant variability in skeletal size and development before the closure of growth plates, and is of low clinical interest in fracture prediction in this age group. The measured sites are adapted from adult practice; vertebral assessment is considered to reflect trabecular bone density and the whole body assessment to reflect the cortical bone. Common fracture sites in children, such as distal forearm and tibia, have been assessed for BMD with DXA and for volumetric BMD with pQCT for research purposes. However, due to lack of sufficient reference data, no other modalities or sites than DXA at the spine, hip, or total body are preferred for clinical use in pediatrics presently.

2.3.3 Primary and secondary osteoporosis

Primary osteoporosis is a rare condition in children. The most common disease is osteogenesis imperfecta (OI), with an incidence of 1:15 000 live births (Stoll et al. 1989). OI is an inherited bone fragility disorder with a wide range of clinical severity; in the majority of cases it is caused by mutations in *COL1A1* or *COL1A2*, the genes encoding the two collagen type I alpha chains (Rauch et al. 2004). Mutations affect the quality and quantity of collagen fibers. OI types I to IV were classified by Sillence et al. before the molecular basis of OI was discovered (Sillence et al. 1979). The most common is type I with mild phenotype: patients present with normal to slightly short stature, bluish sclerae, straight long bones, and predisposition to peripheral and vertebral fractures. Type II is lethal in perinatal period, and type III is severe form with extreme short stature, severe deformities of spine and extremities, and multiple fractures. Type IV is a group of moderately severe, heterogeneous phenotypes not fitting the other types. Recently, new types (V to IX) have been described, mostly with a recessive inheritance pattern, and some with known genetic defect (Michou et al. 2011). No curative treatment is currently available. Bisphosphonates have been shown to increase BMD, improve vertebral shape, and reduce fractures in patients with OI (Rauch et al. 2002b, Land et al. 2006, Cheung et al. 2008).

Idiopathic juvenile osteoporosis (IJO) is a condition with an acute onset of bone pain and walking difficulties in usually prepuberty. It was first described in the German literature by the name "*Pubertätsfischwirbelkrankheit*" (Fish-bone spine disease in puberty) (Catel 1954). The most severe form was later described, in English, by Dent and Friedman under the term IJO (Dent et al. 1965). Vertebral compression fractures are frequent, and metaphyseal fractures may occur, but the disease has a tendency to spontaneous recovery over 2 to 5 years, around the time of puberty. IJO is reported only in approximately 100 children in the literature, and the underlying pathogenesis remains unknown and the natural course poorly characterized (Smith 1995). Other heritable disorders of connective tissue and their genetic causes, as well as the most common causes of secondary osteoporosis in children, and the possible predisposing factors, are presented in Table 3.

Table 3. The most common causes of primary and secondary osteoporosis in children.

Diagnosis	Mechanism
Primary osteoporosis	
Osteogenesis imperfecta	Mutations in *COL1A1* and *COL1A2* (type I−IV), *SERPINF1* (VI), *CRTAP* (VII), *LEPRE1* (VIII), *PPIB* (IX)
Bruck syndrome	Mutations in bone specific telopeptidyl lysyl hydroxylase encoding gene
Osteoporosis pseudoglioma syndrome	Mutations in *LRP5*
Ehlers-Danlos syndrome	Mutations in type V collagen encoding gene
Marfan syndrome	Mutations in fibrillin-1 encoding gene
Idiopathic Juvenile Osteoporosis	Unknown
Secondary osteoporosis	
Neuromuscular disorders	Reduced mobilization, treatment (anti-convulsants etc.)
Chronic illness	Impaired mobility, treatment (corticosteroids etc.)
Leukemia	
Rheumatologic disorders	
Inflammatory bowel diseases	
Solid organ transplantation	
Endocrine and reproductive disorders	Hormonal defect or excess
Impaired nutrition	Reduced muscle mass, calcium or vitamin D deficiency

Gene abbreviations: *COL1A1*, collagen type I alpha 1; *COL1A2*, collagen type I alpha 2; *SERPINF1*, serpin peptidase inhibitor, clade F, member 1; *CRTAP*, cartilage associated protein; *LEPRE1*, leucine proline-enriched proteoglycan (leprecan) 1; *PPIB*, peptidylprolyl isomerase B (cyclophilin B); *LRP5*, low-density lipoprotein receptor-related protein 5.

2.4 Fractures in children

2.4.1 Epidemiology

In the middle of the 20th century, infant and child mortality due to infections and nutritional disturbances decreased rapidly in Europe, and the role of accidents in death rate and morbidity became more important (WHO 1957). Accidents and their causes in childhood were examined in order to develop prevention programs (Ehrenpreis 1957). Fractures are common, comprising 10% to 25% of all pediatric injuries (Engler 1967, Sibert et al. 1981, Landin 1983, Jones et al. 2002a, Mattila et al. 2004, Spady et al. 2004). The first published epidemiological studies on fractures in children date back to the late 19th century. These early reports were hospital-based, as were those larger ones by Beekman and Sullivan from New York, and by Lichtenberg from Nebraska, USA, both of which included over 2000 children with fractures (Beekman et al. 1941, Lichtenberg 1954). The pediatric fractures were found to be common and of patterns different from those of adults (Buhr et al. 1959). Landin pioneered in the field of population-based fracture epidemiology in children. In a large study on 8682 fractures from Malmö, Sweden, over 30-year period, he reported a detailed age- and sex-related pattern of fractures of various types. Landin also observed a doubling of incidence in all pediatric fractures from 1950's onwards, reaching 212 per 10 000 in the late 1970's (Landin 1983). The group later reported a 9% decrease in the overall incidence of pediatric fractures over the following 15 years (Landin 1997, Tiderius et al. 1999). Many groups have assessed the pattern and frequency of fractures in children in hospital cohorts or based on discharge registers, and fewer using the population-based overall incidences. Increasing incidence has been reported for Umeå (in Sweden) and Estonia (Maasalu et al. 2009, Hedström et al. 2010) but no change for some other countries (Cheng et al. 1999, Cooper et al. 2004).

The overall risk of sustaining a fracture during childhood is reported to be almost 50% for boys and 30% for girls (Landin 1997, Cheng et al. 1999, Lyons et al. 1999, Brudvik et al. 2003). The incidence of children's fractures varies with age, season of the year, climate, cultural and environmental factors, and, to some extent, between countries and areas within countries. Boys and girls have a similar, slightly rising, incidence up to age 10 years; thereafter, boys fracture their bones more often than girls: in total, two thirds of the childhood fractures occur in males. The fracture incidence declines rapidly after teen-age years, and is low in adults. After menopause, the fractures start to increase again in women surpassing the childhood's incidence of girls at the age 70. In older men, an increase in incidence is seen later, at age 80 years, although the incidence does not reach the peak of boys in puberty (van Staa et al. 2001) (Figure 7).

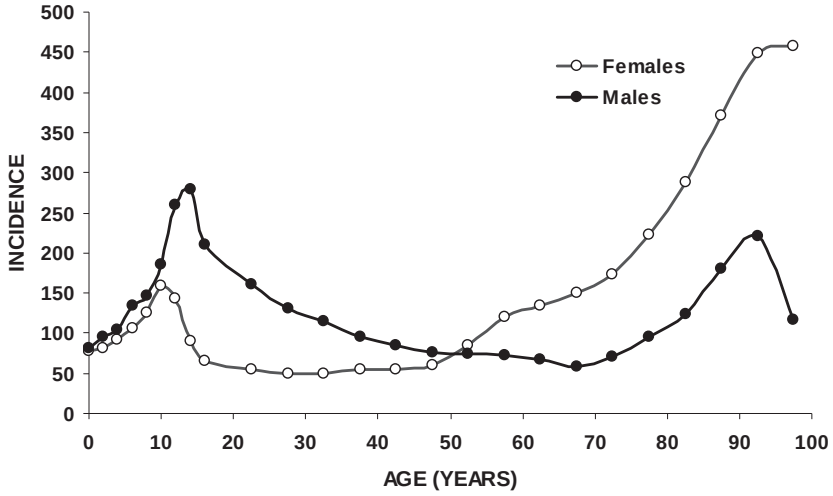


Figure 7. Age- and sex-specific incidence of fractures at any site in children and adults in England and Wales, UK. The figure provides rates per 10 000 person-years, based on total over 300 000 fractures registered to General Practice Research Database, 1988 to 1998. Adapted and combined data from van Staa et al. 2001 and Cooper et al. 2004.

In Finland, fractures have been reported to comprise 13 to 26% of all pediatric injuries requiring physician's attention (Louhimo et al. 1969, Vahvanen et al. 1980, Honkanen 1984, Mattila et al. 2004), and fractures are also the leading cause for hospitalization after injury in childhood (Suominen et al. 2011). Population-based studies in fractures in Finnish children are scarce; the incidence has been similar to that of other Scandinavian countries (Tiderius et al. 1999, Lyons et al. 2000).

2.4.2 Fracture pattern

Most of the fractures in children are located in the upper extremities, 20% in lower extremities, and less than 5% in axial skeleton and trunk. Fracture pattern is age-specific. The most commonly fractured site in children is the forearm (lower arm, antebrachium, radius and ulna), comprising at least one third of all fractures (Landin 1983, Kopjar et al. 1998, Lyons et al. 1999, Brudvik et al. 2003, Cooper et al. 2004, Rennie et al. 2007). Forearm injuries occur most often at the distal end, are as common in girls as in boys, and the incidence increases with the age. Also common in childhood are the fractures of hand, clavicle, and foot, all with a rising incidence with advancing age. Upper arm (humerus) fractures comprise 10% of all pediatric fractures with a bimodal pattern with age; distal humerus fractures are most common in children from 4 to 9 years, whereas the proximal humerus is more commonly injured in older children (Landin 1983, Rennie et al. 2007).

Table 4. Review of studies on fracture incidence in children.

Authors	Country	Years	Fractures	Incidence per 10 000			Age
			n	boys	girls	total	years
Engler 1967	Czechoslovakia	1959-1964	10 672	-	-	178	0-14
Louhimo & Gripenberg 1969	Finland	1967	1 704	-	-	159	0-14
Landin 1983, Landin 1997	Sweden	1975-1979	4 621	257	165	212	0-16
Vahvanen & Wallgren 1980	Finland	1978	1 444	-	-	181	0-14
Honkanen 1984	Finland	1983	1 561	234 -	156 -	192 199	0-14 0-15
Kopjar & Wickizer 1998	Norway	1992-1995	2 477	139	117	128	0-12
Tiderius et al. 1999	Sweden	1993-1994	1 673	235	149	193	0-16
Lyons et al. 1999, Lyons et al. 2000	UK Norway Sweden Finland	1996	2 463 1 075 285 190	444 - - -	275 - - -	361 170 153 176	0-14
Cooper et al. 2004	UK	1988-1998	84 129 *	162	103	133	0-18
Brudvik et al. 2003	Norway	1998	1 725	290	200	245	0-16
Rennie et al. 2007	UK	2000	2 198	239	157	202	0-15
Maasalu et al. 2009	Estonia	1998-2007	-	259-428	155-261	-	0-14
Hedström et al. 2010	Sweden	1993-2007	10 327	-	-	208	0-16

* Number of children with fractures

Lower extremity fractures in pediatric patients are usually of lower leg (tibia and fibula) and foot; the fractures of upper leg (femur) in children are rare (less than 2%). Vertebral fractures are a similarly rare entity in children: they account for less than 2% of all fractures and are commonly associated with high-energy traumas. Half of the spinal fractures occur in the cervical spine, particularly in younger children, and are associated with high mortality. Compression of the vertebral body is the most common type of spinal fracture in the thoracolumbar area (Clark et al. 2001, Newton et al. 2010, Puisto et al. 2010). In children, open fractures and multiple fractures affect small (both about 3%) numbers of patients.

As forearm fractures present with the highest rate in pediatric populations, they are also the most studied ones. Site-specific studies have shown forearm fractures to increase significantly over the last decades in many countries (Tiderius et al. 1999, Hagino et al. 2000, Khosla et al. 2003).

2.4.3 Etiology of fractures

Accidents leading to fractures

The most common type of injury leading to a fracture in children is a fall: on the same level or from low heights comprising together over half of the accidents (Landin 1983, Rennie et al. 2007, Schalamon et al. 2011). In older children, sport-related fractures account for one third of the causes. High-energy traumas are rare in childhood: traffic accidents involving motorized vehicle are the cause in about 5% of the fractures, and falls from greater height are even scarcer. Moreover, bicycle accidents are common in children, and result often in forearm fractures (Vitale 2010). Seasonal variation is remarkable in Scandinavian countries; variation is best seen in traffic accidents as well as for different sport related injuries (Landin 1983, Brudvik et al. 2003, Cooper et al. 2004, Rennie et al. 2007, Hedström et al. 2010). Commonly practiced sports can affect the pattern of fractures to a great extent: soccer related injuries in the UK account for a great part of all traumas leading to fractures (Lyons et al. 1999), whereas skiing accidents have an impact on injury pattern in countries with snowy winter (Landin 1983, Brudvik et al. 2003, Schalamon et al. 2011).

Child abuse as a cause of fractures in young children has been recognized in reports already in the 19th century. Caffey characterized the phenotype with multiple long-bone fractures of different age, and subdural hematomas in 1946 (Caffey 1946). Kempe et al. reported non-accidental trauma to be a major cause of fractures in infants in 1962, and the condition was named battered-child syndrome (Kempe et al. 1962). In a review of fractures occurring in the first year of life, McClelland and Heiple (McClelland et al. 1982) found that at least 44% were of documented nonabusive and accidental etiologies. McClelland also reported 23% of these fractured infants to have some generalized conditions possibly predisposing them to fractures. Non-accidental trauma-related fractures might appear in children of any age but are most common in infants; all types of fractures have been reported in the child abuse literature. However, children who have been physically abused represent a small proportion of the total number of childhood fracture patients. In patients with multiple fractures, or injuries in non-ambulatory subjects or at sites in discordance with child's developmental stage, maltreatment should be considered as a potential cause (Kemp et al. 2008). Several medical conditions can also predispose to multiple fractures in young children, and the differential diagnosis is important but sometimes difficult (Jenny et al. 2006).

Risk factors

Boys fracture more than girls. This has been speculated to be mainly due to their increased exposure to injury risks in their daily activities (Morrongiello et al. 2007). Fractures show a linear increase with age: in all populations, adolescents sustain more fractures than pre-school children. Pubertal children fracture the most – this is likely due to relative undermineralization of adolescent skeleton (Fournier et al. 1997, Bailey et al. 2000) as well as to increased participation in sport activities.

Bone strength is a function of the material properties of bone, often estimated by BMD and bone size, or cross-sectional bone geometry. Previous fractures, or low BMC or BMD, are associated with increased fracture risk also in children of both sexes (Landin et al. 1983, Goulding et al. 2001, Jones et al. 2002b, Goulding et al. 2005a, Goulding et al. 2005b, Clark et al. 2006a, Clark et al. 2006b, Yeh et al. 2006, Cheng et al. 2009). In a prospective study by Goulding et al. from New Zealand, the lower total body areal BMD was a predictor of a new fracture over a 4-year follow-up period in a cohort of girls aged 3 to 15 years. A previous forearm fracture was also an independent risk factor for sustaining a new fracture (Goulding et al. 2000). One previous fracture doubled the risk for a new fracture during childhood, and the risk was three-fold after the second fracture (Goulding et al. 2005b). In a study of 501 healthy participants of 4 to 30 years in the Netherlands, a significantly lower total body BMD was seen in participants who had had a previous fracture. Lower lumbar spine BMD was associated with a fracture history in females, and BMD Z-score seemed to be a good predictor for future fractures in both sexes (Boot et al. 2010). Smaller bone cross-sectional area and smaller cortical area have both been shown to be risk factors for forearm fracture (Skaggs et al. 2001, Kalkwarf et al. 2011).

Childhood growth influences peak bone mass attainment and is associated with fractures later in life (Cooper et al. 1997, Cooper et al. 2001). Overweight children are reported to be prone to injuries and fractures, while normal body fat content seems to be beneficial for bone health in growing children (Skaggs et al. 2001, Goulding et al. 2005a, Rana et al. 2009, Ryan et al. 2010, Viljakainen et al. 2011b). Physical activity has a dual effect on fracture risk. Both low and high physical activity levels are associated with increased risk; probably the first by impaired bone health and the latter by increased injury risk (Ma et al. 2003, Clark et al. 2008). Low calcium intake has also been shown to be associated with impaired bone health: children who avoid milk had lower BMD levels and more reported fractures (Black et al. 2002, Goulding et al. 2004, Chevalley et al. 2005).

In adults, a large meta-analysis on sufficient vitamin D serum concentrations with beneficial bone effects have shown that at least 75 nmol/L is needed; the BMD increased linearly with higher 25-OHD levels up to 80 nmol/L, and a fracture prevention by 25% was reached at levels of > 90 nmol/L. Sufficient blood concentrations are achieved on population level only with daily intakes of D_3 from 700 to 1000 IU (17.5 to 25 µg); the needed supplementation depends to some extent on the starting intake and serum 25-OHD levels (Bischoff-Ferrari et al. 2004, Bischoff-

Ferrari et al. 2006). In children, the sufficient level of serum 25-OHD is still under debate. Low 25-OHD values are shown to associate with lower BMD Z-scores in children with primary or secondary osteoporosis (Bowden et al. 2008). A concentration below 50 nmol/L (20 ng/mL) is commonly considered as hypovitaminosis D or vitamin D insufficiency, and below 37.5 nmol/L (15 ng/mL), vitamin D deficiency (Misra et al. 2008). Severe deficiency, 25-OHD below 12.5 nmol/L (5 ng/mL), is strongly associated with rickets.

2.4.4 Differences between children and adults

Children have higher incidence and different patterns of fractures than adults; proximal femur ("hip") fractures and vertebral fractures are rare in children. Further, the immature bone has lower mineral density, thinner cortices, and the periosteum is relatively thicker and stronger, leading to bone that is more elastic. Incomplete fractures, such as torus fractures of metaphyseal bone, and bowing or greenstick fractures of the diaphysis of long bones, are not seen in mature skeleton. Growth plate at the ends of long bones is a vulnerable place for injury: cartilaginous matrix is softer than the mineralized bone, and joint ligaments are relatively strong in children. Growth plate (epiphyseal) fractures are most common just before skeletal maturity, and consist of 22% of all pediatric fractures (Vitale 2010). Injury to the epiphyseal area can result in premature complete or incomplete closure of the growth plate, with possible growth arrest or deformity subsequently. This problem is more pronounced in the long bones of lower extremities, and growth arrest may require surgical correction (McFarland 1931, Compere 1935, Langenskiöld 1981). On the other hand, fractures resulting in residual angular deformities of a tubular bone may undergo spontaneous correction by remodeling in the diaphysis and metaphysis, and by asymmetrical growth at the epiphyseal plates (Ryöppy et al. 1974). Fracture healing is a process, which starts with hematoma that is turned into mineralized bone. The normal structure and shape of the bone is achieved by remodeling. Healing is faster in children than in adults due to higher degree of remodeling activity: a fracture repair can speed up bone formation, resulting sometimes in overgrowth of bones (Xian et al. 2010).

3 Aims of the study

Epidemiological study is the basis for prevention and allocation of resources. It has been speculated that the incidence of fractures in children is increasing; however, population-based studies are scarce. A low-energy long-bone fracture, and, more so, frequent fractures or a fractured vertebra, can be the first sign of impaired overall bone health in a child. The aim of this thesis was to study fracture epidemiology in children and to widely assess bone health in children with fractures. In addition, it was aimed at evaluating the clinical use of two rarely used diagnostic methods in pediatric patients.

The specific aims of this study were:

1. To obtain epidemiologic and demographic data on fractures in Finnish children and to evaluate changes in the incidence over time
2. To assess bone health in children with a significant fracture history
3. To evaluate the diagnostic accuracy of a low-radiation modality, vertebral fracture assessment (VFA), in children
4. To evaluate the usefulness of histomorphometry in the assessment of skeletal health in children with suspected primary osteoporosis

4 Patients and Methods

4.1 Patients

Study I was conducted to obtain population-based epidemiological data on fractures in children. This cohort included 1373 children who were living in Helsinki, under 16 years of age, and treated for an acute fracture during a 12-month study period starting in February 16, 2005.

The participants for **Study II** were recruited from the hospital cohort of 1412 children who were treated for an acute fracture at the Children's Hospital, Helsinki, during the 12-month period for Study I. All apparently healthy children older than four years, with a clinically significant history of fractures, were recruited. This included patients with a history of at least one low-energy vertebral fracture, or frequent low-energy fractures of long bones; three or more fractures in children under 16 years, or two fractures in children under 10 years. Patients with known diagnosis of primary or secondary osteoporosis, or medication or illness affecting bone, were excluded. Seventy-one (5%) of the children with an acute fracture in 2005 fulfilled the inclusion criteria and were regarded as fracture-prone children; 66 (93%) of the families consented to participate in the study.

The patient group in **Study III** consisted of 65 children with suspected primary or secondary osteoporosis, for whom densitometric spine images and standard spinal radiographs had been obtained for the assessment of vertebral deformities. The group was a combination of children with suspected or diagnosed secondary osteoporosis (n=43) due to solid organ transplantation (n=14), juvenile idiopathic arthritis (n=9), chronic gastrointestinal disease (n=7), or some other chronic illness (n=13). Twenty-two of the children were presumed to have primary osteoporosis: they were assessed in the clinic for a history of frequent low-energy fractures (n=12) or low BMD (n=8); two of them had a clinical diagnosis of OI.

Study IV comprised 24 children on whom a transiliac bone biopsy was performed for suspected severe primary osteoporosis from 2005 to 2007.

Four of the patients from Study II were also in Study III, and fourteen were in Study IV. Six of the patients in Study III were also enrolled to Study IV. Three children were included in Studies II, III, and IV.

Table 5. Number of patients, age range (in years), and the main inclusion criteria in Studies I–IV.

Study	N of patients	Age	Inclusion criteria
I	1373	0−15	Acute fracture during the 12-month study period, living in Helsinki
II	66 *	4−15	Acute fracture during the 12-month study period and a significant fracture history
III	65	4−17	Densitometric spine images obtained for suspected primary or secondary osteoporosis
IV	24	6−16	Bone biopsy for suspected primary osteoporosis

*controls (n=64) in Study II were obtained from previous study on schoolchildren (Viljakainen 2011b).

4.2 Methods

4.2.1 Data collection and study design

We conducted a prospective study during a 12-month period (from February 16, 2005; henceforth, this period is referred to as "year 2005") at the Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland. All patients with an acute fracture and aged under 16 years were recorded. To attain population-based data, all children treated for a fracture during the same period in other regional public clinics treating injured children in Helsinki were also recorded. Altogether 1830 children aged under 16 years were diagnosed with an acute fracture during the 12-month study period. Within the institutions treating children, the first medical contact was at Children's Hospital in 994 (54%), at City Health Centre in 726 (40%, minor traumas), at Otorhinolaryngology clinic in 81 (4%, facial traumas), and at Töölö Trauma Hospital in 29 (2%, high-energy accidents and cranial injuries). Children's Hospital received referrals from other institutes, and treated 1412 patients in total ("hospital cohort"). Out of all 1830 children with fractures, 75.0% (n=1373) were inhabitants of Helsinki city and were included in Study I ("population-based cohort"). Patients' age, sex, home county, date of diagnosis, location of the fracture(s), trauma mechanism, and history of previous fractures were recorded. Personal identification numbers and injury dates were used to confirm that every child was registered only once for each injury. Previous reports on fractures in Helsinki pediatric population were obtained from published reports for 1967, 1978, 1983, and 1994 (Louhimo et al. 1969, Vahvanen et al. 1980, Honkanen 1984, Kuisma 1994). The size of population was obtained for each year from Statistics Finland.

Patients (n=66) for Study II were recruited from the Children's Hospital emergency, wards, and outpatient clinics (n=1412, "hospital cohort"). They were all apparently healthy, had all sustained a traumatic fracture during the 12-month period starting from February 16, 2005, and based on the interview of guardians, they had a clinically

significant fracture history. Children younger than four years were excluded for technical and ethical reasons (sedation needed for DXA). All children with primary or secondary underlying disease or medication known to affect the bone (OI, n=5; meningomyelocele, n=2; dermatomyositis, n=1; leukemia, n=1; terminal liver disease, n=1; and Turner syndrome, n=1), were also excluded. Seventy-one children fulfilled the inclusion criteria, and 93% of the families consented to the study.

For each patient aged 6 to 16 years in Study II, one age- and sex-matched control subject was selected. The control subjects (n=64) were apparently healthy, and had participated in a study on bone health in schoolchildren carried out in Helsinki (Viljakainen et al. 2011b). In Study II, the patients were clinically examined in their visit at the Metabolic Bone Clinic, on average 6 months after the last fracture. Study protocol for the controls was similar for biochemistry and DXA examinations; the analysis and measurements were performed with the same equipment. Life-style habits, and control subjects' and family members' fracture history were assessed with questionnaires.

Patients for Studies III and IV were obtained from the Metabolic Bone Clinic; they were all referred to the Children's Hospital for bone health evaluation for suspected osteoporosis based on fracture history or low BMD at DXA. DXA measurements, imaging studies and blood and urine samples were obtained as part of standard evaluation during the clinic visits, and data were retrospectively collected from hospital records for these studies. For Study III, 65 children with both standard radiographs of the spine and densitometric spine images were registered. Children with various backgrounds were included: they ranged in age from 4 to 17 years, and both sexes, as well as both primary and secondary osteoporosis, were represented. Bone biopsy was performed for clinical reasons in children with unknown bone fragility severe enough for consideration of bisphosphonate treatment. The suspicion of severe osteoporosis in these children was based on a) low BMD (Z-score below -2.0) or b) history of at least three peripheral fractures or at least one vertebral fracture, due to low-energy trauma. All the consecutive 24 patients with bone biopsy in Children's Hospital from March, 2005 to June, 2007, with no known underlying disease or secondary cause, were included in the Study IV.

4.2.2 Fractures (I – IV)

All the radiographs of injured children in Helsinki public clinics during the 12-month study period were primarily assessed by the reviewing staff radiologist on duty, and reanalyzed for Study I (M.Mäyränpää), digitized images were used (AGFA ImPacs System, Agfa-Gevaert, Mortsel, Belgium). The final diagnosis of the acute fracture was based on both clinical findings and imaging. In uncertain cases, the follow-up records, radiographs, and clinical patient history were evaluated. For the diagnosis of a nasal fracture, clinical confirmation by the physician at the Department of Otorhinolaryngology was regarded as sufficient without radiographic confirmation. Fractures were classified using the 10th version of International Classification of Diseases (ICD-10, www.who.int): each fracture was recorded separately, with the

exception of simultaneous fractures of radius and ulna of one forearm, of tibia and fibula of one lower leg, of multiple vertebrae, or multiple digits or metatarsal/carpal bones, which were recorded as one fracture. Refractures, defined as fractures at one site occurring within a year, were recorded as separate fractures.

Fracture history and details of the possible trauma mechanisms were recorded after patient and parental interview for the children treated at Children's Hospital. For the children diagnosed at other institutes the data were collected from patient charts. Trauma energy was considered high in all accidents involving motorized vehicle (i.e., car, bus, motorcycle, or all-terrain vehicle). In addition, whenever the patient's injury resulted from a fall from 3 meters or more of height, or from an equivalent level of trauma, the trauma was defined as high energy. Other traumas were regarded as low-energy injuries.

No criteria for clinically significant fractures were available in 2005. In this work (Study II), significant fractures were defined as fractures of vertebrae or long bones of the extremities, resulting from low-energy trauma. Long-bone fractures included fractures of clavicle, upper arm (humerus), forearm (antebrachium), and metacarpals (= upper extremity), and those of upper leg (femur), lower leg (tibia and fibula), and metatarsals (= lower extremity). Fractures of the digits, ribs, nose, or skull, pathological fractures including refractures, and fractures from birth traumas, and small bony avulsion particles were not considered as significant fractures in this study. Fracture history, including the last one diagnosed during the study period in 2005, and all possible fractures experienced prior to that, was available for 96% of the children treated at Children's Hospital.

4.2.3 Clinical data (II, III, IV)

Height and weight were recorded during clinical assessment (Studies II, III, and IV). Height was measured with a Harpenden stadiometer and weights with electric scale. Heights were transformed into Z-scores and weights into height-adjusted values (weight %) using age- and sex-specific references for Finnish children (Sorva et al. 1990, Pere 2000). Body mass index (BMI) was calculated [weight/ (height x height)] and expressed in kg/m^2; BMI Z-scores were calculated using international age- and sex-specific reference data provided by WHO (www.who.int) (II and IV). Pubertal maturation was clinically assessed by physician, according to Tanner (Tanner 1962) (II and IV).

The patients and parents were interviewed at the clinical visit for any underlying diseases or conditions affecting bone health (Study II). Dietary habits, history of vitamin D supplementation, and average daily calcium intake, as well as level of physical activity of the children, were assessed by detailed parental interview. National recommendations were used to define sufficient daily intake of calcium for age: 600 mg for children under 6 years, 700 mg for 6–9 years, and 900 mg for children 10 years or older (The National Nutrition Council 2005 / the Ministry of Agriculture and Forestry,

www.ravitsemusneuvottelukunta.fi). Physical activity was assessed by interview: average amount of leisure-time activity or organized sport per week, in hours, was recorded. All physical activity of longer than 15 minutes at a time was included: free play outdoors, school trips by bike or walking, and physical education at school. Physical activity level was considered high if weekly hours of active exercise or play were seven or more (II).

4.2.4 Biochemistry (II, IV)

Blood and urine samples were collected in Studies II and IV to assess bone metabolism and overall health. Samples were obtained before noon. Complete blood count, plasma concentrations of calcium (Ca), phosphate (Pi), and alkaline phosphate (ALP) were measured by standard methods. Serum concentrations of 25-hydroxyvitamin D (25-OHD) were determined by high-pressure liquid chromatography followed by UV detection (HP 1100 Liquid Chromatograph, Agilent Tech., Santa Clara, CA, USA) (Turpeinen et al. 2003). 25-OHD below 50 nmol/L was regarded as consistent with hypovitaminosis D, and below 37.5 nmol/L as consistent with vitamin D deficiency (Misra et al. 2008). Plasma parathyroid hormone (PTH) was measured by solid-phase enzyme-labeled chemiluminescent immunometric assay (IMMULITE 2000, DPD, Diagnostic Products Corporation, Los Angeles, CA, USA). Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (in girls over 8 years) and testosterone (in boys over 10 years) were measured by standard assays to exclude hypogonadism. Anti-tissue transglutaminase antibody (tTGAbA) level was measured to screen for celiac disease (II). Urine samples were analyzed for calcium, phosphate, and creatinine levels. Bone formation marker procollagen type I N-terminal peptide (PINP, µg/L) and the resorption marker collagen type I C-terminal telopeptide (ICTP, µg/L) were measured from serum by radioimmunoassay (UniQ, Orion Diagnostica, Espoo, Finland).

Table 6. Biochemical parameters for calcium homeostasis and bone turnover used in this thesis.

Parameter		Reference	Units	Study II	Study IV
P-Ca	Calcium	age-specific	mmol/L	X	X
P-Pi	Phosphate	age-specific	mmol/L	X	X
P-ALP	Alkaline phosphatase	age- and sex-specific	U/L	X	X
S-25-OHD	25-hydroxyvitamin D	≥ 50	nmol/L	X	X
P-PTH	Parathyroid hormone	8–73	ng/L	X	X
S-PINP	Procollagen type I N-terminal peptide	age-specific	µg/L	X	X
S-ICTP	Collagen type I C-terminal telopeptide	age-specific	µg/L	X	X
U-Ca/U-Crea	Calcium to creatinine ratio	≤ 0.7	mmol/mmol	X	X
U-Pi/U-Crea	Phosphate to creatinine ratio	≤ 3.5	mmol/mmol	X	

P-, plasma. S-, serum. U-, urine.

4.2.5 Skeletal imaging (I, II, IV)

For the fracture diagnosis in Study I, radiological examinations were carried out as part of normal care, and the diagnosis was based on standard radiographs or CT of the injured site. Presence of rickets or bone dysplasia was excluded by evaluating plain radiographs of the left side long bones (hemi-skeleton) (II). Radiographs of the left hand were used to determine bone age according to Greulich and Pyle (Greulich et al. 1959) (II, IV). Scoliosis was defined as a Cobb's angle 20 degrees or more from the spinal radiographs taken for the study, or if previously diagnosed by orthopedist.

4.2.6 Vertebral assessment (II – IV)

Anterior-posterior and lateral radiographs of the thoracic and lumbar spine were obtained in supine position, to identify vertebral deformities (II, III, and IV). The visibility and diagnostic accuracy of vertebral fracture assessment (VFA) by densitometer scan was evaluated in Study III; the whole-spine images (Discovery A, Hologic, Bedford, MA, USA), both in anterior-posterior and lateral view, were used to assess vertebral morphometry. Standard radiographs of the spine were used for comparison. All vertebral bodies in Th4 to L5 were evaluated for visibility and deformities by two independent clinicians, who were blinded to the patients' clinical data. Compressions were classified according to grading by Mäkitie et al. (Mäkitie et al. 2005) and by semiquantitative technique by Genant (Genant et al. 1993). For discrepant readings, consensus reading was performed on standard radiographs with very good interobserver agreement for the presence of fractures (Cohen kappa 0.98). Furthermore, semiautomatic computer-aided fracture assessment application (Physician's Viewer, version 4.0, Hologic, Bedford, MA, USA) was used for evaluation of vertebral morphology; the shape of each vertebra is analyzed by using six-point vertebral height measurements and calculating percentual deformities based on these measurements. The grading in this application is based on the semiquantitative technique (Genant et al. 1993).

4.2.7 Densitometry (II – IV)

Bone mineral content (BMC) and bone area were measured with dual-energy X-ray absorptiometry (DXA) to obtain areal BMD (aBMD) for lumbar spine, total hip (hip and proximal femur), and whole body. Measurements were performed with Hologic Discovery A (Hologic, Bedford, MA, USA), using pediatric software version 12.4. Body composition assessment included whole body fat percentage, lean body mass (LBM), total mass, and bone area (II). The aBMD and fat percentage values were transformed into Z-scores using age- and sex-specific reference data for the equipment. The BMD Z-score was corrected for bone age if delayed or advanced more than one year (II, IV). BMD Z-scores at or below -2.0 were regarded as abnormally low for age at all measured sites.

4.2.8 Bone biopsy (IV)

A full-thickness transiliac bone biopsy sample was obtained from 24 children with suspected severe primary osteoporosis in Study IV. A double-labeling course, with 10-day interval period and with per oral tetracycline, was introduced three weeks prior to the procedure. Bone samples were collected 3 or 4 days after the last labeling period.

The biopsies were performed as outpatient procedure in an operating room at the Children's Hospital, all by the same operator (M. Mäyränpää). The procedure required general anesthesia in all but two adolescent patients, on whom only local anesthesia was used. The transiliac biopsy was performed from a standardized location of anterior superior iliac spine as described by Recker (Recker 2008). Biopsy specimen was drilled manually with a specific bone needle of inner diameter 5 mm (in 6 patients) or 7.5 mm (in 18 patients). The patient was placed in supine position with a small rise under the pelvis and the ipsilateral knee, and with the ilium and umbilicus exposed; the anterior ilium was cleaned draped. The biopsy site, located 2 cm posterior to the anterior-superior iliac spine, was identified; lidocaine was used to anesthetize the skin, subcutaneous tissue, and periosteum of the iliac bone. A vertical skin incision of 2 to 3 cm was then made by scalpel. The underlying muscle and fascia were separated by blunt dissection until the lateral iliac periosteum was exposed; it was then incised and pushed aside. The pointed trochar was inserted through the outer guide sleeve and then inserted through the skin incision, then applied firmly to the exposed bone, pointing toward the umbilicus. The outer guide was rotated until firmly implanted and anchored to the lateral ilium. At this time, the pointed trochar was withdrawn and the trephine inserted through the outer guide. The trephine was rotated clockwise with a steady moderate pressure until the trephine had advanced through the full depth of the ilium. The trephine was then removed, and the blunt extractor inserted through the top of the trephine, gently pushing out the bone core specimen. To control hemostasis, bony defect was packed with absorbable hemostat (Surgicel® Fibrillar, Ethicon, Somerville, NJ, USA), skin incision was closed with sutures in layers, and the site covered by a pressure dressing for 24 hours.

Bone histomorphometry

The bone biopsy specimen was placed in 70% ethanol, then gradually dehydrated and embedded in polymethyl metacrylate. A special microtome was used for sectioning (Polycut S, Cambridge Instruments GmbH, Nussloch, Germany). A modified Masson-Goldner trichrome stain was used for light microscopy in sections of thickness of 3 µm. Unstained sections (thickness of 10 µm) were prepared for polarized light and fluorescence microscopy. Trabecular bone was identified from randomly selected sections, excluding a distance of at least 50 µm from the endocortical surface to avoid cortical or endocortical bone to be measured. Structural, static, and dynamic parameters were analyzed using a semiautomatic image analyzer (Bioquant Osteo, Bioquant Image Analysis Corporation, Nashville, TN, USA). Nomenclature, abbreviations, and standard formulas following the recommendations of the American Society for Bone and Mineral Research were used (Parfitt et al. 1987).

The pediatric age-specific data for transiliac bone histomorphometry published by Glorieux et al. (Glorieux et al. 2000) were used as reference for all parameters and the results were transformed into Z-scores. Only for eroded surface, a different reference was used: the eroded surface (ES/BS) was determined with a method described by Recker et al. and Rehman et al. and commonly used in adults, and values above 10% were considered abnormal for all ages (Recker et al. 1988, Rehman et al. 1994). In the present study, bone volume was regarded low for age if bone volume per tissue volume (BV/TV) was below -1.0 SD. The interpretation of turnover activity was based on formation parameters osteoid surface (OS/BS) and osteoblast surface (Ob.S/BS), and resorption parameters osteoclast surface (Oc.S/BS) and eroded surface (ES/BS); a cut-off value of +1.0 SD was used for OS/BS, Ob.S/BS, and Oc.S/BS, and a value of 10% for ES/BS. Turnover was regarded high if both formation parameters were high (above +1.0 SD), both resorption parameters were high, or at least one formation and one resorption parameter was high. Low turnover was determined by two formation parameters (OS/BS and Ob.S/BS) below -1.0 SD, or immeasurably low mineral apposition rate (MAR).

Table 7. Methods used in this thesis.

Method	Original publication
Fracture history	I, II, III, IV
Clinical assessment	II, III, IV
Biochemistry	II, IV
Radiography	II, III, IV
Bone densitometry	II, III, IV
Vertebral assessment with densitometry	III
Bone biopsy and histomorphometry	IV

4.3 Ethical considerations

The study protocol was approved by the Research Ethics Board of Helsinki University Central Hospital (Dnro. 442/E7/2004). The participants were fully informed about the study, they attended it on a voluntary basis, and the use of the collected information for medical research was explained to them. An informed written consent for participation was obtained from parents, and assent from children for the bone health assessment study (II), in accordance with the Declaration of Helsinki. Bone biopsies (Study IV) and spinal radiographs (Studies III and IV, and partly in Study II) were obtained from children for clinical reasons. Bone biopsy was performed under general anesthesia in all but two adolescent patients, who agreed to the use of local

anesthesia with an option for conversion to general anesthesia. The study subjects were informed about the findings of the examinations, and, if needed, further referred for appropriate care.

4.4 Statistics

Age- and sex-specific fracture incidences were calculated by dividing the number of patients and fractures during the 12-month study period by the number of inhabitants in the same age group, as reported for January 1, of each year studied. Numbers were obtained from Statistics Finland (www.stat.fi). All fracture incidences are given per 10 000 inhabitants. Results for height, BMI, BMD, bone resorption and formation markers and for bone histomorphometry parameters were expressed as Z-scores, SD units from age-specific or age- and sex-specific means. Differences between two groups were tested for significance using Student's t-test or Mann-Whitney U test, as appropriate. Analysis of variance (ANOVA) or Kruskal-Wallis test was applied to compare more than two groups. Categorical variables were tested with Chi-square test. Associations between variables were tested with Pearson correlation analysis or Spearman's rank correlation. Statistical analyses were performed using Microsoft Office Excel 2003 and PASW Statistic software SPSS (Versions 13.0, 17.0, and 19.0. SPSS, Inc., Chicago, IL, USA) Cohen kappa was used for interobserver accuracy (StatXact 7, Cytell Inc., MA, USA). All tests were two-tailed, and $p<0.05$ was considered statistically significant throughout the study.

5 Results

5.1 Fracture epidemiology in children (I)

5.1.1 Fracture incidence

Altogether, 1396 fractures in 1373 children were recorded for children in Helsinki in 2005. Within this cohort of children under 16 years, the median age was 10.8 years (11.6 for boys, 9.8 for girls), and the average age 10.0 years (10.6 for boys, 9.1 for girls). The overall fracture incidence for children under 16 years was 163 per 10 000 for both sexes. Fractures were more common in boys (63%, male to female ratio 1.6:1). The annual fracture incidence was 201/10 000 for boys, and 124/10 000 for girls. Estimated risk for at least one fracture during childhood is 27% for boys and 16% in girls. The sex difference was only seen in children older than 10 years (boys 327 vs. girls 147/10 000, $p<0.001$); equal incidences of fractures were seen in younger children (116 vs. 104 per 10 000, $p=0.62$). In puberty, both sexes had a peak in the fracture rate: girls reached the highest incidence (263/10 000) at 10 years, and boys three years after (386/10 000). (Figure 8).

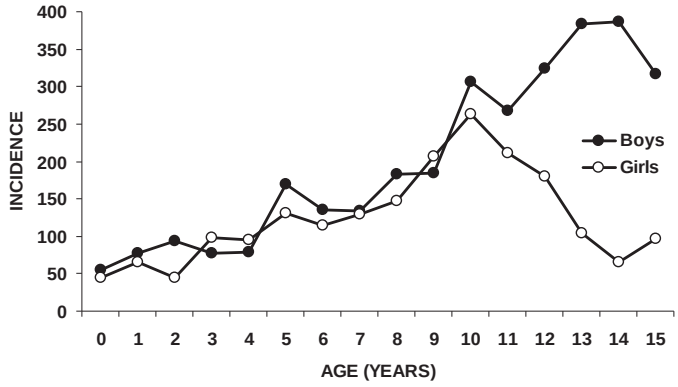


Figure 8. Sex-specific incidence of fractures in children in Helsinki, Finland. Year 2005. Incidence per 10 000.

Most of the injuries were registered at the end of summer (August to September) or during the winter months (January to March), these peaks were due to an increase in upper extremity fractures, whereas low-extremity fractures were more constant throughout the year. In weekends, the daily fracture rate rates were lower compared with other days (3.3 vs. 4.0 patients/day, $p=0.008$).

5.1.2 Long-term trends

There had been a 24% increase of pediatric fractures in Helsinki from late 1960's through to the 1980's: the overall fracture incidences in Helsinki for children 14 years and younger were 159, 181, and 196 per 10 000 for years 1967, 1978, and 1983, respectively (Louhimo et al. 1969, Vahvanen et al. 1980, Honkanen 1984). Overall incidence for children under 16 years was 199 in 1983 and 180 in 1994 (Honkanen 1984, Kuisma 1994). The present study found an annual incidence of 163 for children under 16 years and 160 for those under 15 years. Thus, an 18% decrease in overall fracture incidence was observed between 1983 and 2005, and this declining trend was already observed in 1994 (Figure 9). The most significant decrease was seen in children aged 10 to 11 years (-23%, $p<0.0017$) and 12 to 13 years (-33%, $p<0.0001$) (Figure 10). The decrease in overall fracture incidence was equal for both sexes: 17.5% for boys and 19.7% for girls. No change was seen in seasonal variation.

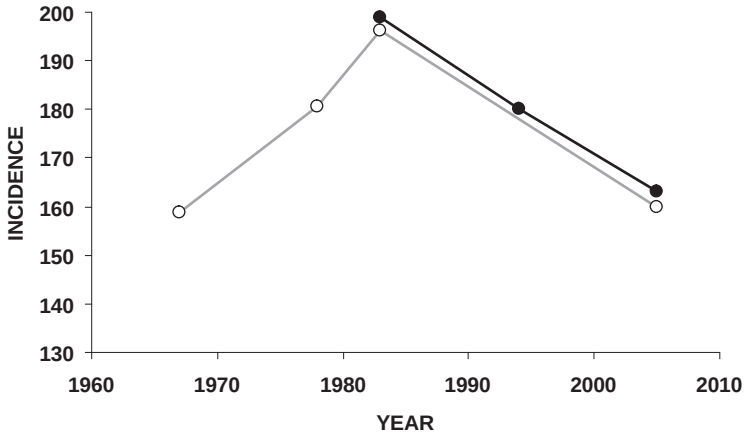


Figure 9. Change in overall incidence of fractures in children in Helsinki, Finland. Incidence per 10 000, for children under 15 years with open circles, and for children under 16 years with black dots (data for years 1967, 1978, 1983, and 1994, from Louhimo et al. 1969, Vahvanen et al. 1980, Honkanen 1984 and Kuisma 1994, respectively; 2005, the present study).

5.1.3 Traumas leading to fractures

Trauma history was available for 98.8% of the 1373 patients treated in Children's Hospital. Most of the injuries leading to fractures in children were low-energy accidents: high-energy accidents including motorized vehicle accidents and falls from heights (>3 meters) comprised only 2.3% of all the traumas. This proportion was unchanged from the last report 22 years earlier. Falling down when running or walking (21%) or from less than standing height (≤1.5 m, 13%) were common. The injury was related to a winter-sport activity (16%) as often as to a summer-specific activity (11%). Bicycle accidents were also common (5%). Trauma was unknown for 1% (n=13) of the patients with a median age 1.0 years; two of these children were previously diagnosed with OI, and two had an underlying disease leading to secondary osteoporosis.

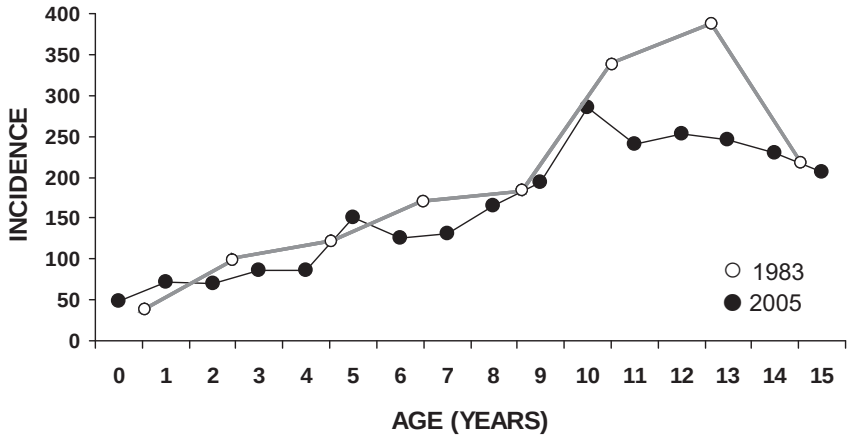


Figure 10. Overall incidence of fractures by age in children in Helsinki, Finland. Year 2005 with black dots and year 1983 with open circles (from Honkanen 1984). Incidence per 10 000. Significant decrease in incidence was seen for children aged 10 to 11 years and 12 to 13 years.

5.1.4 Fracture pattern

Upper-extremity fractures were the most common fracture type, accounting for 73% of the injuries. Fractures were in the forearm in 37% (n=517), the hand in 21% (n=294), and the upper arm in 9% (n=123) of all fractures. Lower-extremity fractures were involved in 22%; the most common were those of lower leg (crus) and foot. Axial fractures (spine and pelvis), and fractures of the head (skull and face), were much less common (1.4% and 3.7%, respectively). Detailed fracture pattern is presented in Table 8. Male dominance was seen for all fracture sites where more than one fracture was diagnosed; over 75% male rate was seen only for metacarpals and for the humerus diaphysis. In the age group 10 to 14 years, where boys had higher fracture incidence than girls, significant difference was observed for fractures of the clavicle, forearm, hand, and foot. Fracture incidence of forearm, hand, lower leg, and foot were positively associated with advancing age: skull fractures occurred in small children and upper arm fractures mostly in children 4 to 6 years old (Figure 11). Most common fractures occurring to young children were those of clavicle, distal humerus, or metatarsals.

Forearm fractures were 68% more common in boys than in girls (75 vs. 45 per 10 000). The median age for forearm fractures was 10.7 years (mean 10.2 ± 3.6 years). The incidence of forearm fractures peaked at 13 years in boys and at 10 years in girls. Patients with forearm fracture had suffered from low-energy traumas: only 1%

were injured in traffic and 1% in falls from three meters or higher. The most common accidents leading to forearm fracture was falling when running or walking (22%) or falls from lower heights (23%). Winter-related activities (figure skating, snowboarding, sledge riding, ice hockey, or alpine and Nordic skiing) were reasons for the trauma in 22%, and summer-related activities (bicycle riding, swinging, soccer, skateboarding, horse riding, roller skating, or trampoline) in 23%.

Table 8. Distribution of fractures in population-based cohort (n=1373 children, Study I) and in the cohort of fracture-prone children (n=66 children, Study II). Incidence per 10 000.

	Population-based data (I)			Fracture-prone children (II)	
	n	Incidence	%	n	%
Face	33	3.9	2.4	0	0
Skull	17	2.0	1.2	0	0
Spine	16	1.9	1.1	12	6.2
Pelvis	4	0.5	0.3	1	0.5
Clavicle	89	10.4	6.4	35	18.1
Humerus (upper arm)	123	14.4	8.8	17	8.7
Antebrachium (forearm)	517	60.4	37.0	89	45.6
Hand	294	34.3	21.1	18	9.2
Femur (upper leg)	24	2.8	1.7	2	1.0
Patella	7	0.8	0.5	0	0
Crus (lower leg)	127	14.8	9.1	14	7.2
Foot	142	16.6	10.2	7	3.6
Others	3	0.4	0.2	0	0
Total	***1396***	***163***	***100***	***195***	***100***

Compared with the previous epidemiological studies, there has been a steady rise in forearm fractures in the pediatric population in Helsinki: +47% from the report in 1967 to 2005 (Figure 12). Over the preceding 22 years, the incidence has increased 31%, from 46.2 (in 1983) to 60.4 (in 2005) per 10 000 inhabitants. Forearm fractures were more common in males (+50%) and in females (+24%), and an upward trend in the incidence was seen for school-aged children and younger (7 to 14 years, +31%, under 7 years, +47%) (Figure 13). Fractures of the upper arm showed a slightly increasing incidence in all age groups, resulting in a 39% increase, from 10.6 in 1983 to 15.0 in 2005. All other anatomical sites presented with stable or decreasing incidence. The decrease was the greatest in hand and foot fractures (39% and 48%, respectively), and was mostly due to significant reduction in the age group of 10 to 14 years (-30%) (Figure 13).

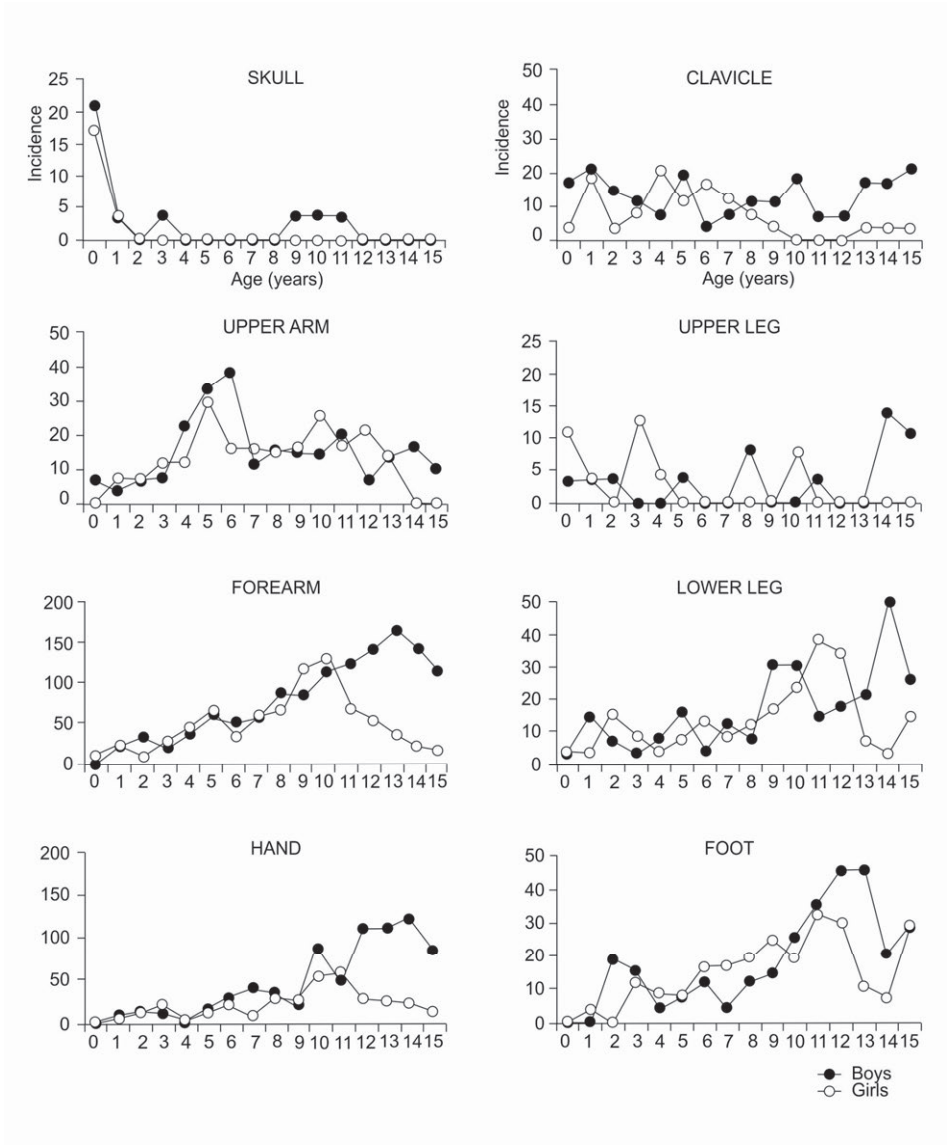


Figure 11. Site-specific incidence of fractures by age and sex in children. Fracture incidence in girls with open circles and in boys with black dots. Year 2005, in Helsinki, Finland. Incidence per 10 000. Age-related pattern was observed for skull, forearm, hand, lower leg, and foot fractures. Gender-related differences were seen for forearm and hand fractures.

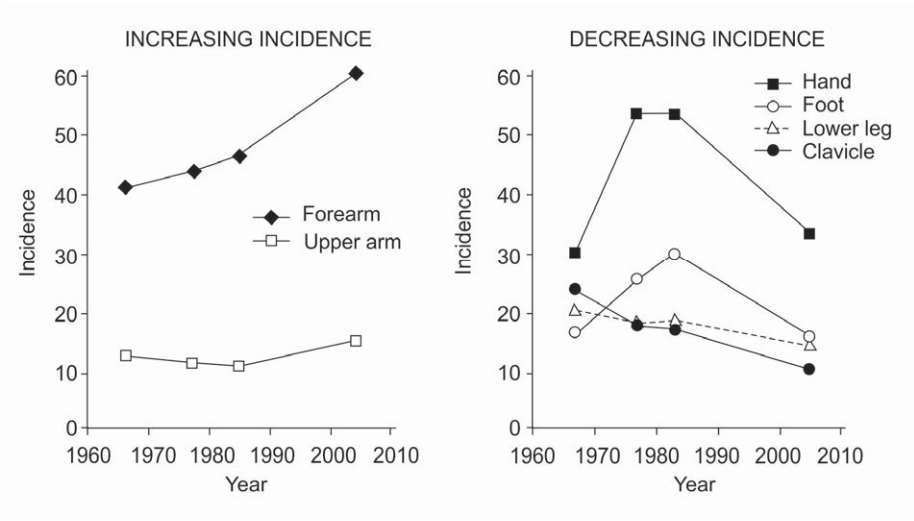


Figure 12. Changing incidence of most common fractures in children over the period 1967 to 2005 in Helsinki, Finland. Incidence per 10 000, children under 15 years. An increasing trend was observed for forearm and upper arm fractures, whereas incidences of fractures of foot and hand rose towards 1983, and both have decreased markedly between 1983 and 2005 (data from Louhimo et al. 1969, Vahvanen et al. 1980, Honkanen 1984, and present study).

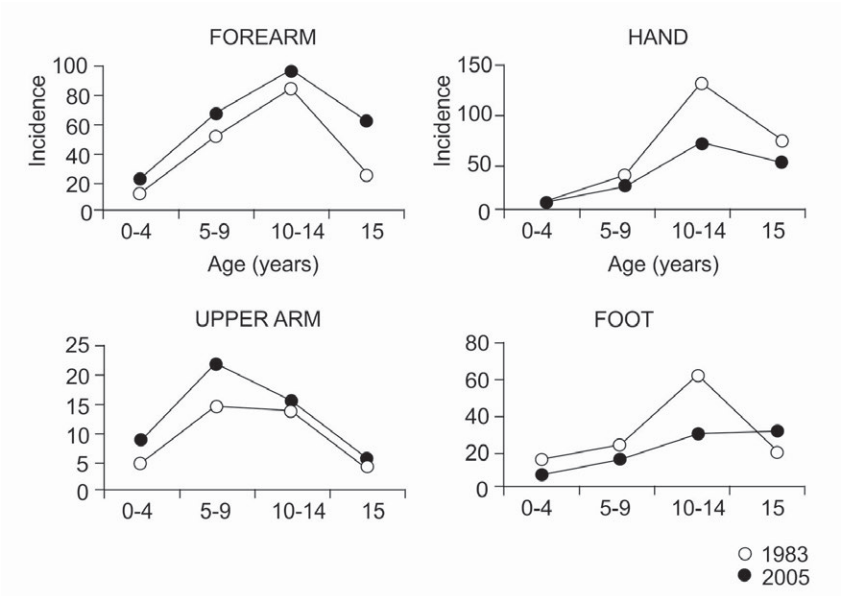


Figure 13. Age-specific change in the incidence of common fractures in children aged 0 to 15 years in Helsinki, Finland. Year 1983 with open circles (Honkanen 1984) and year 2005 with black dots (present study). Incidence per 10 000. For the forearm and upper arm fractures, the increase was independent of patients age. For the fractures of hand and foot, the most marked decrease was observed for the age-group of 10 to 14 years.

5.2 Children with repeated fractures

In the population-based cohort, 23% of the fractured children treated at the Children's Hospital were found to have a history of previous fractures; 5% had suffered more than two fractures prior to their admission in 2005. During the 12-month study period, 0.4% of the children sustained a refracture, most commonly of the forearm. Further, within the same period, 1.9% of the children were treated for two different fractures from two separate injuries. Of the hospital cohort, 77 (14%) patients under 10 years of age had sustained two fractures, and 74 had three or more fractures before 16 years of age (6%). Five of these fracture repeaters were children with diagnosed OI, and three had a known secondary osteoporosis; 11% were younger than 4 years. Of the rest, only 68% had sustained clinically significant fractures.

5.2.1 Characteristics (II)

In a hospital cohort of 1412 children with fractures, 5% were found to have either vertebral fracture or a clinically significant fracture history for age resulting from low-energy traumas, and they were further determined as fracture-prone in this work (II). Children under 4 years of age and those with known disease or medication affecting bone, were excluded from the study. Fracture-prone children (n=66, 44 males) were aged from 4.4 to 16.8 years at the time of clinical assessment; the median age was 10.2 years (Table 9). They were on average of normal height (mean 0.7 ± 1.0 SD, range -1.4 to +2.8 SD) and weight (mean 8.5 ± 17.1%); 7 were obese (BMI over +2.0 SD, max +4.3 SD) and 11 were regarded as overweight (BMI +1.0 to +2.0 SD). All had pubertal development appropriate for their age, and 43 (65%) were prepubertal. One age- and sex-matched control was obtained for each fracture-prone child six years and older (n=64): the groups of patients and controls were equal for age, height, height-adjusted weight, BMI Z-score, and pubertal stage.

Table 9. Clinical characteristics of subjects in Studies II–IV at the time of assessment. Results given as mean ± SD. Age in years.

	Study II			Study III	Study IV
	Patients		Controls		
	Traumatic VFx	Frequent LBFx			
Patients, n	11	55	64	65	24
Males, n (%)	8 (73%)	36 (65%)	43 (67%)	37 (57%)	17 (71%)
Age, mean	11.3 ± 2.0	10.6 ± 3.2	11.2 ± 2.7	12.0 ± 4.0	12.0 ± 2.6
median	11.2	10.0	10.8	12.1	12.0
range	7.7–13.6	4.4–16.8	7.4–16.8	4.6–17.7	6.7–16.6
Height Z-score	0.6 ± 0.9	0.7 ± 1.0	0.5 ± 1.0	-0.8 ± 1.8	0.2 ± 1.2
Weight %	11 ± 20	8 ± 17	6 ± 13	1 ± 12	10 ± 19
BMI Z-score	0.7 ± 1.2	0.5 ± 1.1	0.4 ± 1.1	-0.3 ± 1.2	0.4 ± 1.4

VFx, vertebral fracture(s); LBFx, long-bone fractures; BMI, body mass index

5.2.2 Fracture pattern in fracture-prone children (II)

Patients in Study II had experienced at least one traumatic vertebral fracture (11 patients), two long-bone fractures before age 10 years (26 patients), or at least three long-bone fractures before age 16 years (29 patients) resulting from low-energy trauma. Four of the children with recent traumatic vertebral fracture had no previous fractures; one had sustained another vertebral fracture five years earlier. The remaining six patients had also a history of long-bone fractures (1 to 3 fractures per patient). These twelve cases of traumatic low-energy vertebral fractures in 11 children were usually due to falls from 1−2 meters height (67%), two to skiing-related injuries, one to a soccer game, and one to a trampoline injury.

History of vertebral fractures was one of the inclusion criteria for Study II, and vertebral fractures were five times more prevalent in this cohort as compared to the general population. Similarly, rates of forearm fractures ($p=0.015$) and clavicle fractures ($p<0.001$) were over-represented in the cohort of fracture-prone children; the rate of hand and foot fractures were less than half of the expected number (Table 8). Male dominance, similar to the population-based cohort, was seen also in children with a history of low-energy vertebral fracture(s) (boys 73%), and in children aged 10 to 16 years with frequent low-energy long-bone fractures (boys 77%). The boys to girls ratio of younger children (<10 years) with frequent fractures was more equal (boys 58%), reflecting the equal incidence of fractures in both sexes before puberty (Table 9). The fracture-prone children more often had siblings with fractures than the controls did (30% vs. 13%, $p=0.015$). No difference was seen for the rate of fathers with fractures or mothers with fractures between the groups.

5.3 Life-style factors (II)

The physical activity was assessed as average amount of hours of sports per week, organized or unorganized; active free play outdoors, school trips by bike or walking, and physical education at school were included (II). Fracture-prone children reported a weekly average of 7.2 hours (median 7.0 hours / week, range 2 to 12 h/w) of vigorous physical activity. As compared to age- and sex-specific controls, the physical activity level was lower in the patient group (below 7 h/w in 31% vs. 6%, $p=0.001$). Six of the children (4 males, median age 12.4 years) did not participate in any sports other than those provided at school (2 h/w); 18 (27%) of the fracture-prone children (16 males, median age 11.9 years) reported 10 hours or more of physical activity per week. Highest physical activity level (≥ 10 h/w) was associated with male gender ($p=0.021$), higher calcium intake ($p=0.069$), and higher BMD Z-score at all measured sites. The average BMD Z-score was 0.0 at lumbar spine, +0.4 at hip, and +0.4 at whole body in those with highest physical activity, with at least +0.6 difference in Z-score at all sites as compared to patients with lower physical activity level ($p=0.013$ to 0.022). Higher BMD was also observed for the patients with a physical activity level of 7 hours or more per week; the difference in Z-score was +0.4 for lumbar spine ($p=0.076$) and for whole body ($p=0.023$). Physical activity at ≥ 7 h/w or ≥ 10 h/w level, however, was not a determinant of the number of peripheral fractures or prevalence of vertebral compressions within the study group. Physical activity did not correlate with BMI.

Calcium intake in patients varied from 240 mg to 1800 mg per day (mean 990 mg) and in controls (data available for 50/64) from 690 to 2470 mg per day (mean 1190 mg, p=0.037). Calcium intake was below recommendations for age in 31% (n=20) of the patients, four times more often than in the controls (8%, p=0.002). Two of the patients reported no consumption of dairy products; they were both on calcium supplementation. In patients, low calcium intake was associated with increased age (12.6 vs. 9.9 years, p=0.001), puberty (52% of pubertal vs. 19% of prepubertal patients, p=0.010), and lower whole body BMD Z-score (-0.4 vs. 0.1, p=0.023). Low vitamin D concentrations were more frequently observed with low than with sufficient calcium intake: 80% of the low-intake subjects had serum 25-OHD below 50 nmol/L, and 50% had concentrations below 38 nmol/L. Calcium intake was not related with the prevalence of vertebral compressions, but low daily intake was associated with higher number of peripheral fractures (3.2 vs. 2.6 fractures per patient, p=0.042).

5.4 Biochemistry (II, IV)

Bone health was assessed by blood and urine samples in a group of 66 apparently healthy fracture-prone children and their healthy controls (II), and in 24 children with suspected primary osteoporosis (IV). All patients had normal blood count and plasma levels of calcium and phosphate; pubertal maturation (Tanner stage and hormonal parameters) was appropriate for age in all the patients. One patient was positive for anti-tissue transglutaminase antibody and was diagnosed with celiac disease after intestinal biopsy (II). Hypercalciuria (U-Ca/Crea > 0.7 mmol/mmol) was seen in seven patients (11%) in the fracture-prone group (II) (0.79–1.24 mmol/mmol), and in five patients (21%) in the bone biopsy group (IV) (0.74–0.86 mmol/mmol). Hypercalciuria was more prevalent in the patients than in the controls (6/63 vs. 1/63, p=0.12), and the mean U-Ca/Crea was also significantly higher in the patients than in controls (0.32 vs. 0.19 mmol/mmol, p=0.001). Hyperphosphaturia (U-Pi/Crea > 3.5 mmol/mmol) was observed in 14 patients (21%) in the fracture-prone group, and in only one of the controls (p=0.001); the mean U-Pi/Crea was higher in the patients than in controls (2.43 vs. 1.96 mmol/mmol, p=0.059). In patients, hyperphosphaturia was related to lower age (p<0.001), and higher daily calcium intake (p=0.047). Differences between patients and controls for U-Pi/Crea persisted in subgroup analysis of children less than 10 years of age, and was independent of age and month of sample collection. Hypercalciuria or hyperphosphaturia were not associated with lower BMD or with any histomorphometric parameter.

5.4.1 Bone markers

ALP was slightly supranormal for age (+2.6 to +4.9 SD) in four pubertal children; two in the fracture-prone children (II) and two in children with bone biopsy (IV). ALP was normal in others and in all controls. PINP values over 800 µg/L were observed in 14 patients; values over 1000 µg/L were observed in 9 (for all, range 105.3 to 1719.0 µg/L). Only 7 control subjects had PINP over 800 µg/L; the highest was 1004.9 µg/L. No statistical differences between the fracture-prone children and controls were seen for ALP, PINP, or ICTP. Serum concentrations of bone formation markers (ALP, PINP)

or resorption marker (ICTP) did not correlate with BMD Z-score at any measured site. In the bone biopsy study (IV), cut-off values 800 µg/L (2 patients) or 1000 µg/L (1 patient) for PINP, and 20 µg/L (6 patients) or +2.0 SD (the same 6 patients) for ICTP, were not predictive for specific histomorphometric findings.

5.4.2 Vitamin D

All but one child of the 66 fracture-prone children group had received vitamin D supplementation (official recommendation 10 µg per day in younger than 2 years) as an infant (II). At the time of study assessment, only 5% reported regular vitamin D supplementation throughout the preceding two years. One third had received some irregular supplementation (up to 10 µg per day) during the winter months after age 2 years; the measured 25-OHD was no different in those with wintertime supplementation and others (46 vs. 49 nmol/L, $p=0.34$). Serum 25-OHD samples taken in the summer (from June to October) were on average 14 nmol/L higher than during winter months (mean 56 vs. 42 nmol/L, $p<0.001$). There was a negative correlation between 25-OHD and PTH ($r=-0.28$, $p=0.023$).

Vitamin D concentrations were below recommendations in the majority of the patients: 55% of the fracture-prone children (II) and 58% of the patients with suspected primary osteoporosis (IV) had 25-OHD below 50 nmol/L. None had concentrations below 12.5 nmol, but altogether 28% had vitamin D deficiency (below 37.5 nmol/L), and 25% values consistent with hypovitaminosis (38 to 49 nmol/L). Only 5 (7%) of the total 76 children had 25-OHD above 75 nmol/L. Controls in Study II had serum 25-OHD assessed during the winter-months (November to May); the mean 25-OHD was 43 nmol/L (range 17 to 77 nmol/L), and 69% of the controls had concentration below 50 nmol/L. When the patient results were adjusted for season, no differences were observed for vitamin D levels between the fracture-prone patients and their controls.

5.5 Radiological imaging

5.5.1 Appendicular skeleton (II, IV)

Based on radiographs of the long bones, no skeletal dysplasia or other structural abnormalities were diagnosed in the group of 63 fracture-prone children in Study II. No radiographic evidence of rickets was found in any of the subjects with frequent fractures (II), traumatic vertebral fractures (II), or suspected primary osteoporosis (IV). Bone age, determined from the left-hand radiographs, was normal for 71% of the fracture-prone children (II) and 58% of the children with suspected primary osteoporosis and biopsy (IV). It was delayed in 11% (II) and 17% (IV), and advanced in 18% and 25%, respectively. No significant scoliosis was diagnosed in any (II).

5.5.2 Spine (II – IV)

A total of 117 compressed vertebrae were observed in 46 children altogether in Studies II, III, and IV. Six of the children with altogether 18 vertebral fractures were patients in two studies. Distribution of the compressed vertebrae is presented in the Figure 14. Vertebral fractures were known prior to the assessment in 17% of the fracture-prone children (II), and in 38% of the children with bone biopsy (IV). In addition, children with history of repeated long-bone fractures were found to have asymptomatic vertebral compressions when screened by spinal radiographs: 15% in Study II and 21% in Study IV. The asymptomatic compressions were found in the thoracic region in all but one child; in addition, the thoracic region was the most common site for the known traumatic fractures in the children in all studies.

5.5.3 Vertebral fracture assessment (III)

The visibility and diagnostic accuracy of the densitometer-derived VFA images was evaluated in a group of 65 children with previously diagnosed or suspected osteoporosis (Table 9). The vertebrae in the upper thoracic spine (T4 to T7) had slightly more compromised visibility in VFA images as compared to radiographs (90% vs. 98%), but a good visibility for all the vertebral bodies from T8 downwards was noted for VFA images (96% vs. 99.8%). The visibility was limited by the summations caused by intrathoracic tissues. Children with at least two non-visible vertebrae in VFA images (n=17) were younger (median age 9.6 vs. 12.8 years, p=0.11) and shorter (height 135 vs. 152 cm, p=0.056) than those patients with good visibility. The limited visibility was also associated with the lower BMD at lumbar spine (median BMD Z-score -2.5 vs. -1.3, p=0.011) in these children. The semi-computerized method (using Physician's Viewer) could detect the shape of vertebral bodies in the lumbar area in most of the children. However, due to its poor detection rate in the thoracic area, the overall accuracy was rated good only in 17% of the patients. These patients with good

visibility for the software, were all aged more than 14.6 years, and were of at least 152 cm in height; the median Z-score for lumbar spine BMD was -1.0 (range -2.2 to -0.1).

Altogether, 25 vertebral fractures in 13 patients (20%) were recognized in standard radiographs (Figure 14). The accuracy of VFA in children for vertebral fracture detection was poor: only nine (36%) of the fractured vertebrae seen in standard radiographs were classified as compressed in the VFA images. Others (64%) were classified either as “normal” (8) or as “not visible” (8). Only four of the nine compressions were seen by both observers, and five were regarded as normal by one of the observers. The semi-computerized method could recognize the only fractured vertebrae in the group of children with good visibility; however, several false positives were also reported for these same 11 patients.

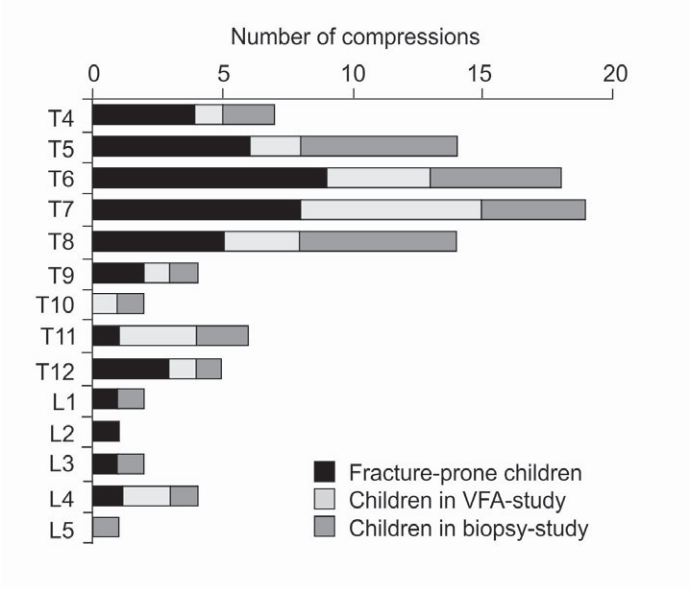


Figure 14. Distribution of thoraco-lumbar vertebral compressions in children, combined data from Studies II, III, and IV. Altogether, 99 compressed vertebrae, traumatic and asymptomatic, were observed in 40 patients. Six of the children with vertebral fractures were patients in two studies, and duplicates (18 compressions) were omitted from the numbers of Study IV in the figure. T, thoracic; L, lumbar; VFA, vertebral fracture assessment.

5.6 Densitometry (II – IV)

BMD Z-score was significantly lower in fracture-prone children as compared with their sex- and age-matched controls at all measured sites (II). For lumbar spine, the difference was 0.8 (-0.5 vs. +0.3, p<0.001); for total hip, the difference was 0.4 (-0.0 vs. +0.4, p=0.007); and for whole body, 0.6 (-0.1 vs. +0.5, p<0.001) (Figure 15). The BMD Z-scores for the whole cohort of fracture prone children (n=66) ranged from -2.8 to +1.8 for lumbar spine, from -2.2 to +2.0 for hip, and from -2.2 to +1.9 for whole body. Subjects enrolled in the study for VFA assessment (III) were patients with diagnosed or suspected primary or secondary osteoporosis. Some of them were markedly smaller than normal for age; their height Z-score median was -0.6 (range -5.0 to +3.1) and BMI Z-score median -0.1 (range -3.2 to +1.8) (Table 9). Mean BMD Z-score at lumbar spine for these children was -1.6 (median -1.8, range -4.3 to +1.1). Patients in Study IV were children with suspected severe primary osteoporosis; bone biopsy was performed prior to possible treatment. These children had on average BMD Z-score at lumbar spine -1.2 (median -1.2, range -3.1 to +1.0).

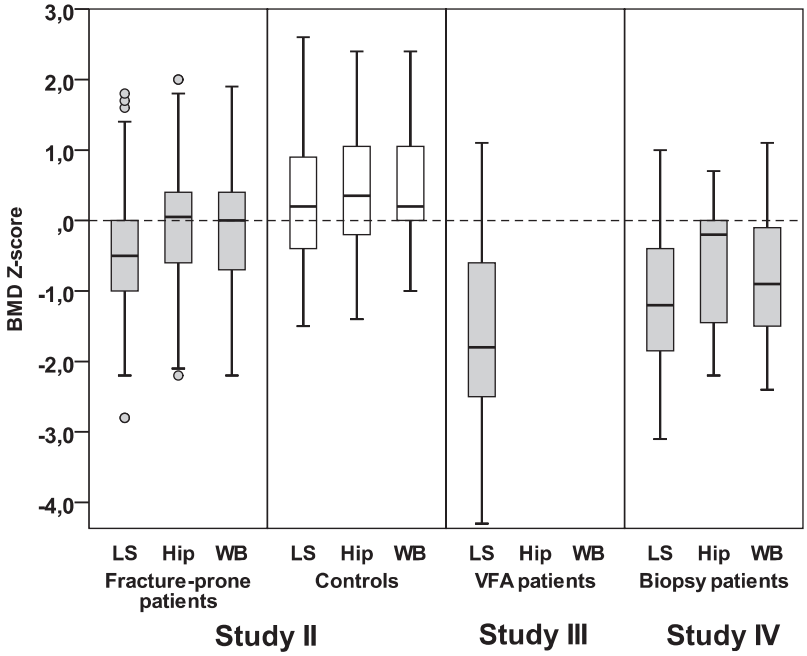


Figure 15. Bone mineral density (BMD) Z-scores in all study subjects. Fracture-prone patients (n=66) and their controls (n=64) (Study II), patients enrolled in the VFA study (n=65) (III), and in the patients in bone biopsy study (n=24) (IV). In Study IV, Z-scores are adjusted for bone-age. LS, lumbar spine; WB, whole body; VFA, vertebral fracture assessment. The bottom of each box indicates the 25th, the cross line the 50th (median), and the top the 75th percentile of the variables. Whiskers extend to the minimum and the maximum within 1.5 times the length of the box. Outliers are shown as dots.

The BMD was positively correlated with vitamin D in both patients and controls at all measured sites; there was a significant difference between three vitamin D groups for whole body (p=0.038) (Figure 16). None of the subjects with normal serum 25-OHD (≥ 50 nmol/L, n=48) had whole body BMD Z-score below -1.0, whereas eight children with low 25-OHD had their BMD Z-score between -1.0 and -2.0, with only one child below -2.0 (p=0.02). Similar findings were present for lumbar spine and hip, but no statistical significance was found. BMD Z-score was below -1.0 at lumbar spine in four patients, and at hip in one with normal 25-OHD. Positive correlations were observed for BMI and BMD at lumbar spine (r=0.393, p=0.001) and hip (r=0.317, p=0.009). No difference, however, was seen for those with BMI Z-score over +1.0 (n=18) and other patients.

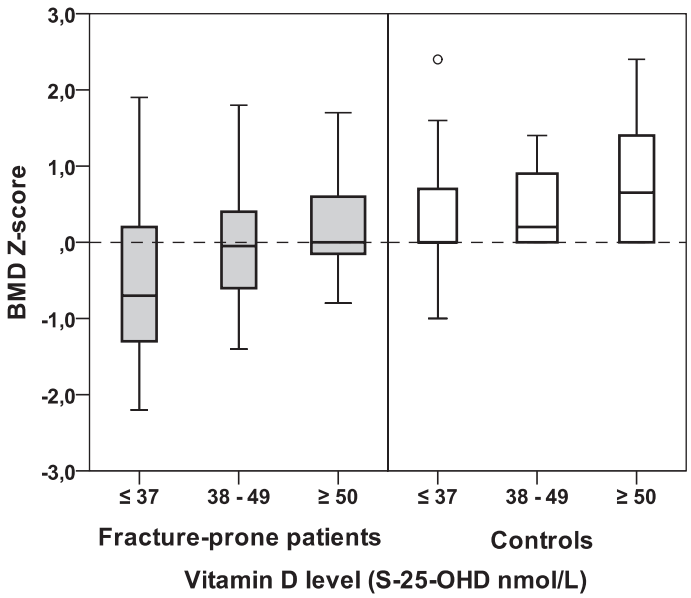


Figure 16. Bone mineral density (BMD) Z-score at whole body in relation to serum vitamin D levels, in patients (n=66) and controls (n=64) (Study II). Serum 25-OHD values below 38 nmol/L regarded as vitamin D deficient, between 38 and 49 nmol/L as hypovitaminosis D, and 50 nmol/L and above are regarded as normal. The bottom of each box indicates the 25th, the cross line the 50th (median), and the top the 75th percentile of the variables. Whiskers extend to the minimum and the maximum within 1.5 times the length of the box. Outliers are shown as dots.

Whole body DXA was analyzed for body composition in fracture-prone children and their controls (II). The groups were equal in age- and sex-adjusted fat percentage (-0.2 vs. -0.3 SD, p=0.52). Lower mean BMC per lean body mass (LBM) was observed for patients (48.43 vs. 51.22 g/kg, p<0.001), but no difference was seen in bone area in relation to LBM (Area/LBM 66.12 vs. 66.32 cm2/kg, p=0.91).

5.7 Bone biopsy and histomorphometry (IV)

Bone biopsy was obtained from 24 children with suspected severe primary osteoporosis, based on frequent fractures and/or low BMD (Table 9). Technically the bone samples were of good quality; two cortices and trabecular bone were present in all. The first bone specimen was macroscopically broken in two (a second sample was obtained) and trabecular bone was partially broken in specimens of two subjects with thin trabeculae. One patient had transient nerve symptoms attributable to iliohypogastric nerve compression during the procedure and one superficial skin infection was treated with oral antibiotics.

Results of histomorphometric parameters were transformed into age-specific Z-scores. Bone volume (BV/TV) Z-score ranged from -3.5 to +2.2 in the whole cohort (Table 10). Out of the 24 patients, seven (29%) patients had low bone volume for age; their mean BV/TV was -1.8 (median -1.5, range -3.5 to -1.2). The remaining 17 were found to have normal bone volume in histomorphometric analysis; mean BV/TV was +0.1 (median +0.1, range -1.0 to +2.2) (Figure 17). Bone turnover was regarded as high in seven patients and low in seven patients; the remaining 10 had normal turnover. Low bone volume was not associated with any specific bone turnover state: both bone volume and turnover results were independent of patient's age, sex, BMI, or Tanner stage.

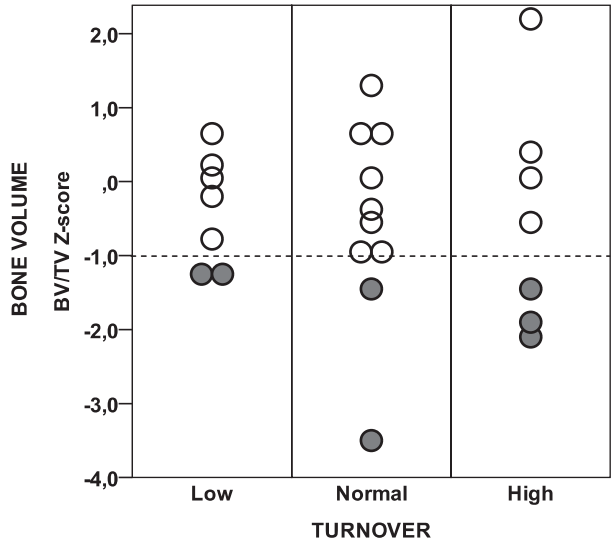
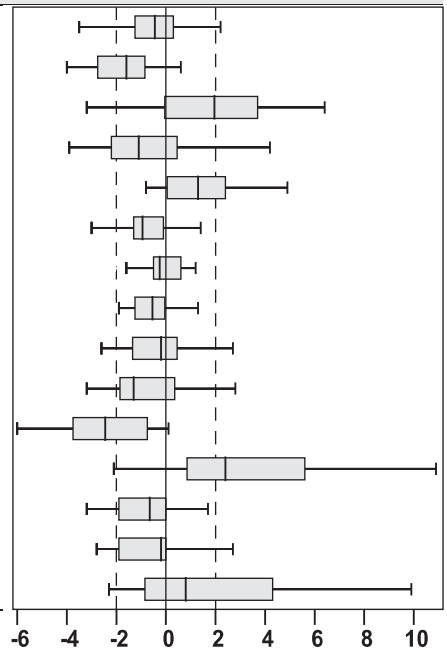


Figure 17. Bone biopsy findings in 24 children with suspected severe primary osteoporosis (Study IV). Histomorphometry analysis revealed an equal number of patients with low (7), and high turnover (7), and normal in 10. Low bone volume, determined as BV/TV Z-score < -1.0, was also found in each turnover group (total n=7, black dots).

Table 10. Histomorphometric results in the 24 children with suspected primary osteoporosis in Study IV. Normal values: BV/TV Z-score > -1.0, others mean ± 2.0 SD; normative data from Glorieux et al. 2000. For Z-score distribution, the bottom of each box indicates the 25th, the cross line the 50th (median), and the top the 75th percentile of the variables. Whiskers extend to the minimum and the maximum within 1.5 times the length of the box. Outliers not shown.

Parameter	Z-score median (range)	Within normal limits %	Z-score distribution
BV/TV *	-0.4 (-3.5 to 2.2)	63	
Tb.Th	-1.6 (-4.0 to 0.6)	63	
Tb.N	2.0 (-3.2 to 11.5)	50	
Tb.Sp	-1.1 (-3.9 to 4.2)	63	
O.Th	1.3 (-0.8 to 6.8)	67	
OS/BS	-0.9 (-3.3 to 3.0)	83	
OV/BV	-0.2 (-2.3 to 3.4)	83	
Ob.S/BS	-0.6 (-1.9 to 6.2)	92	
W.Th	-0.2 (-4.5 to 9.0)	75	
MS/BS	-1.3 (-3.2 to 6.4)	63	
MAR	-2.7 (-6.0 to 0.1)	38	
Mlt	2.4 (-2.1 to 20.5)	25	
BFR/BS	-1.5 (-3.2 to 3.4)	75	
Ac.f.	-0.6 (-2.8 to 2.7)	79	
Oc.S/BS	0.8 (-2.3 to 19.4)	50	



Eroded surface (ES/BS) was increased (> 10%) in four (median 5.8%, range 1.01 to 12.77%). The bone formation rate (BFR/BS) correlated with turnover status. BFR/BS was lowest in the low turnover group and was decreased in average to 14% of the value found in reference subjects (Z-score mean -2.4 ± 0.7). BFR/BS was also low in patients in the normal turnover group (56%, -1.2 ± 0.9). BFR was only normal in the high turnover group (119%, +0.4 ± 2.0) (p=0.018, Kruskal-Wallis test).

A trend for positive correlation was seen in BV/TV with BMD Z-score, although no statistical significance was reached at any measured site (e.g., lumbar spine r=0.338, p=0.106). Out of the five patients with low BMD for age in DXA (Z-score below -2.0), only three had BV/TV below -1.0 SD. On the other hand, low bone volume by histomorphometry, seen in 7 patients, was associated with lower BMD Z-score at lumbar spine, although the difference was not statistically significant (-1.9 vs. -1.0 SD, p=0.087) (Figure 18). No differences were seen for hip (-1.0 vs. -0.5 SD, p=0.28) or total body (-1.2 vs. -0.7 SD, p=0.32). Low turnover by histomorphometry was

associated with higher BMD at lumbar spine (p=0.011), and a trend was seen for whole body (p=0.065) as compared to those with high or normal turnover. Higher turnover was associated with low serum 25-OHD; highest BFR/BS was also seen in vitamin D deficiency group (p=0.003) (Figure 19).

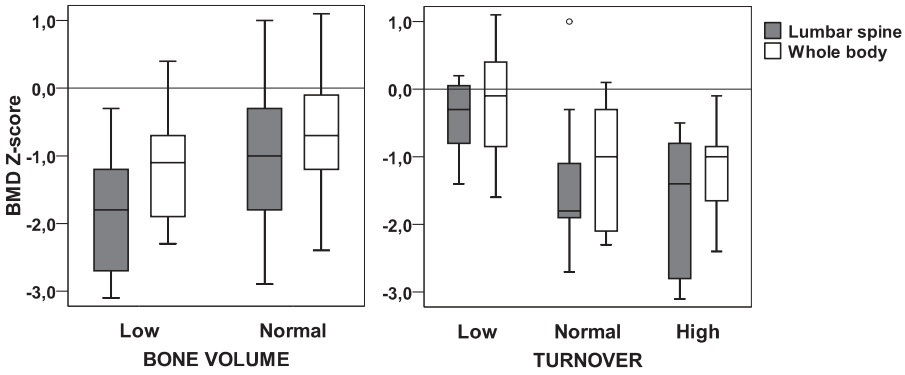


Figure 18. Bone mineral density (BMD) in children with suspected severe primary osteoporosis (Study IV). BMD Z-score at lumbar spine (grey) and whole body (white). Subjects (n=24) are grouped by bone volume in histomorphometry (left panel) and by turnover (right panel). No statistical significance was observed for any differences. The bottom of each box indicates the 25th, the cross line the 50th (median), and the top the 75th percentile of the variables. Whiskers extend to the minimum and the maximum within 1.5 times the length of the box. Outliers are shown as dots.

Osteoid thickness (O.Th) ranged from 5.3 µm to 13.1 µm, and mineralization lag time (Mlt) ranged from 8 days to 76 days, and was more than 25 days in 11 patients (46%) (Table 10). Those three patients with both O.Th greater than 9 µm and Mlt more than 25 days, indicating possible osteomalacia, had serum 25-OHD concentrations not different from the others (29, 38, and 71 nmol/L, respectively).

The effect of low vitamin D concentration was observed for structural and formation parameters (Figure 19). Patients with vitamin D deficiency (S-25-OHD below 37.5 nmol/L, n=9) had significantly higher MS/BS (p=0.006), BFR/BS (p=0.003), OS/BS (p=0.01), and OV/BV (p=0.012) and thicker trabeculae (Tb.Th p=0.01), as compared to those with 25-OHD 38 nmol/L or above (n=15). Hypovitaminosis D (S-25-OHD below 50 nmol/L, n=14) was similarly associated with higher OS/BS (p=0.031), OV/BV (p=0.022), and greater Tb.Th (p=0.007) as compared to those with normal vitamin D status. No statistical significance was reached for BV/TV, ES/BS, or Oc.S/BS by vitamin D concentrations. Thus, in histomorphometric analysis, the vitamin D levels were inversely associated with turnover: patients with low serum concentrations of 25-OHD (≤ 37 nmol/L) had more often high turnover (5/9) than those with normal 25-OHD (1/10). No difference was detected in bone volume by vitamin D concentrations.

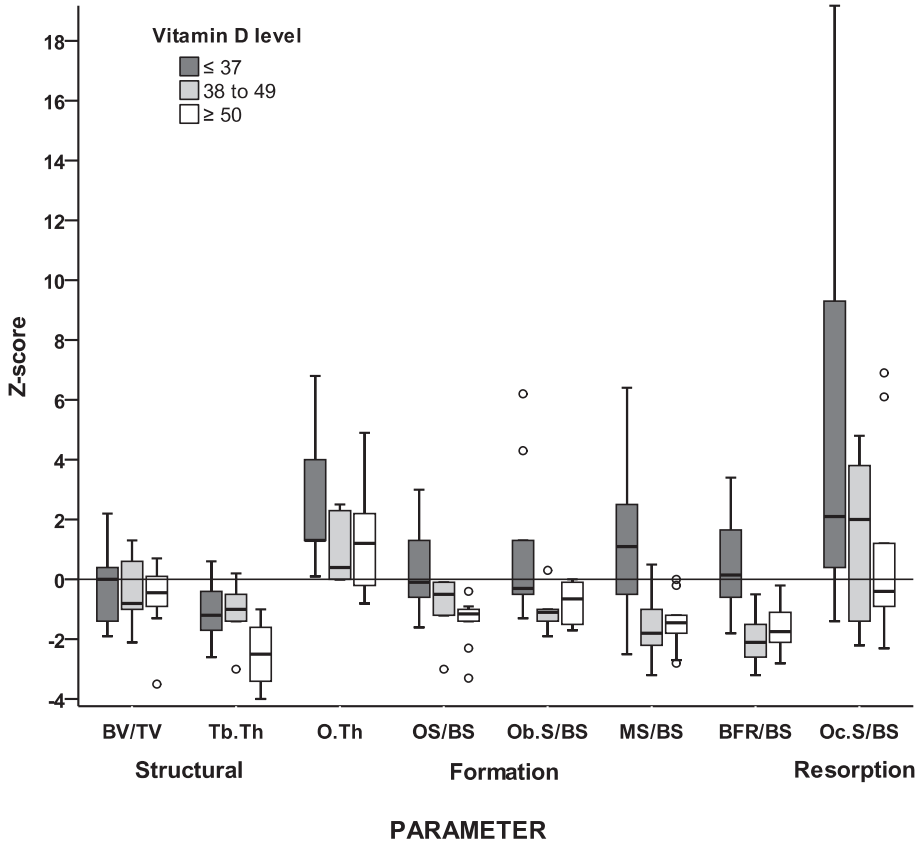


Figure 19. The effect of low vitamin D concentration was seen for structural and formation parameters. Significant differences were observed for Tb.Th, OS/BS, and OV/BV, with S-25-OHD cut-off both at 37.5 nmol/L and 50 nmol/L; and for MS/BS, and BFR/BS, with S-25-OHD cut-off at 37.5 nmol/L. The bottom of each box indicates the 25th, the cross line the 50th (median), and the top the 75th percentile of the variables. Whiskers extend to the minimum and the maximum within 1.5 times the length of the box. Outliers are shown as dots.

Histomorphometric findings were used to determine the proper treatment in patients with suspected severe primary osteoporosis. Based on the DXA results and fracture history, all these 24 children were candidates for bisphosphonate treatment; however, it was not considered necessary in 14 with normal bone volume on biopsy. Bisphosphonate treatment was assigned to six. All had vertebral compressions and peripheral fractures; BMD Z-score at lumbar spine was below -2.0 in four. Low 25-OHD, absence of vertebral compressions, normal BMD in DXA, and/or low turnover in histomorphometry led to more conservative treatment (calcium and vitamin D supplementation, and clinical follow-up) in four, despite the low bone volume in biopsy.

6 Discussion

Fractures are common in children, and an increase in the incidence has been suspected. Underlying disease or life-style factors can influence bone health in children, and the risk for fractures might be modifiable. The diagnostic criteria of osteoporosis or the criteria for when to assess overall bone health in children without secondary causes have not been established. The present study was intended to obtain epidemiologic data on childhood fractures in Finland; a 12-month survey was performed in all public health institutions in Helsinki, Finland. All children with fractures were recorded for trauma and history of fractures; population-based incidence was obtained for children under 16 years. Further, a subgroup of apparently healthy children with frequent fractures or history of a vertebral fracture was assessed for bone health and risk factors for fractures. The usefulness of two methods that have not been widely used in children, vertebral fracture assessment from densitometer-derived images and transiliac bone biopsy, were evaluated in children with suspected osteoporosis.

6.1 Fractures in children

6.1.1 Overall epidemiology

Up to the 1970's, the goal of epidemiological studies in children was to identify the most common bones to break and the types of pediatric fractures. The landmark of epidemiological studies has been that by Landin, with its detailed age- and sex-specific pattern of pediatric fractures and their long-term trends in Sweden (Landin 1983). The natural process of fracture healing has also been studied in order to improve treatment strategies. Today, the goal is to gather data on a specific geographical area and on specific age groups, in an attempt to establish preventive programs. In our study, we were able to obtain data for a large urban city of Helsinki. During a 12-month study period, almost 1400 fractures were recorded in children aged 0 to 15 years. The annual incidence of all pediatric fractures for Helsinki inhabitants (163 per 10 000) was 18% lower than that of two decades ago. This overall decrease was similar to that reported in Sweden 10 years earlier (Tiderius et al. 1999), and could reflect the current economic situation and changes in life-style in these two Nordic countries.

Fractures in Helsinki decreased in both sexes over the preceding 22 years, and in particularly in the age group of 10 to 14 years. The study assessed neither the socio-economical background of all the fractured children nor their growth data. However, other studies have demonstrated an increased polarity and a decrease in mean leisure-time activity, and also an increase in the proportion of overweight teenagers over the same time in Finland (Samdal et al. 2007, Vuorela et al. 2009).

In all epidemiological studies, as in this work, the fracture rate increases strongly at the time of peak growth velocity. This may be due to relative undermineralization of the adolescent skeleton (Bailey et al. 1989, Fournier et al. 1997) as well as to increased participation in sport activities, as the higher bone mass associated with increased physical activity in children does not seem to compensate for the increased exposure to injuries (Clark et al. 2008). Boys tend to have a behavior more prone to injuries than girls do, and boys attend sports with increased physical risk (Morrongiello et al. 2007). The rise in estrogen levels leads to excess bone mineral accrual relative to muscle strength in pubertal girls: it is proposed that pre-menopausal women have more bone relative to their mechanical needs than males do (Schoenau et al. 2000). The enhanced bone strength (by mechanical stimulus) in boys is challenged during the faster and longer growth spurt period in puberty. Thus, the phenomenon of more fractures with increasing age, predominantly in males, is somehow natural and expected; we should be more concerned about the frequent fractures in younger age group, where repeated fractures are more likely to reflect impaired bone health.

Few population-based data are available regarding the overall epidemiological pattern of childhood factures during longer observation periods; most information comes from Scandinavian countries (Landin 1983, Honkanen 1984, Tiderius et al. 1999, Brudvik et al. 2003, Hedström et al. 2010) and from UK studies derived from the General Practice Research Database (Cooper et al. 2004, Rennie et al. 2007). Hospital-based reports rely on the referrals or cover smaller population samples, and interview-based studies may have a selection bias and smaller coverage. Some epidemiological works are based on populations up to 18 years of age, reasonable in the sense that fractures in males stay high until age 17. On the other hand, cohorts including children less than 14 years of age represent the true childhood type of fractures in both genders. In this work, we decided to analyze fractures in children under 16 years of age: thereafter, the regular sports training, frequency of alcohol abuse, and risk taking behavior all have a significant role in fracture risk (Mattila et al. 2007). There were also previous data available on fracture epidemiology in Helsinki for this age group; further, age 16 is the upper age limit for acute patients admitted at Children's Hospital, where this work was carried out. Although results from epidemiological studies may not be directly applicable to other countries due to the great geographical variation, based on the observations in this work for Helsinki and by Tiderius for Malmö, Sweden (Tiderius et al. 1999), a true decrease in the incidence of overall fractures is likely to be occurring. Further, the frequently-reported forearm fracture increase is a simultaneous event.

6.1.2 Forearm fractures

In previous reports from 1960's to 1980's, fractures of the hand were the most common fracture types in children in Helsinki. However, over the past 40 years, there has been a steadily rising incidence of pediatric forearm fractures from 1967 to 2005. In this study, for year 2005, forearm fractures present with the highest incidence (60.4 per 10 000) and constitute 37% of all fractures in children under 16 years. The current incidence was 47% and 31% higher than in 1967 and 1983, respectively. Tiderius

reported a similar incidence of forearm fractures (62 per 10 000) and increase preceding 1993−1994 in Sweden, but this increase was only seen in girls (Tiderius et al. 1999). Also in the USA, the increase was significantly greater in females (Khosla et al. 2003); on the contrary, an increase was only seen for males in Japan (Hagino et al. 2000). In Helsinki, we observed an increase in forearm fractures in both genders: 50% in boys and 24% in girls. Whereas the decrease of hand and foot fractures in our study was related to pre-adolescents, the increase of forearm fractures was similar in all age groups. In two other studies, the rise in distal forearm fracture has been reported to be more pronounced in pre-adolescents, from 10 to 14 years (Khosla et al. 2003, de Putter et al. 2011).

The increase in forearm fracture incidence is of significant importance, as forearm fractures constitute of major part of all fractures treated with surgical operations. Although the number of fatal injuries has declined dramatically in Finland, probably due to improved traffic safety, the incidence of fractures leading to hospitalization has increased steadily (Parkkari et al. 2000). In particular, the incidence of the upper extremity fractures in the hospitalized children, and even more, the rate that is treated operatively, is rising (Helenius et al. 2009, Sinikumpu et al. 2012). Children with forearm fractures have been shown to have lower bone mass and BMD, a greater percentage of body fat, and less calcium intake than their peers without a history of fracture, demonstrating a link between these fractures and deficient bone health (Goulding et al. 2005a, Clark et al. 2006a, Clark et al. 2006b, Ryan 2010).

6.1.3 Vertebral fractures

In the population-based study, traumatic vertebral fractures comprised a small minority of all fractures in children (1%); only in one third had vertebral fractures resulted from high-energy traumas. Some were from winter-activity injuries, and others were due to falls from 1−2 meters height.

Vertebral compressions without known preceding back pain or back injury (asymptomatic) were observed in radiographic assessment in 15% of children evaluated for repeated long-bone fractures. Acute traumatic and asymptomatic vertebral compressions were both found mostly in the thoracic spine. All but one child with asymptomatic compressions were under 10 years of age; no child with a history of four long-bone fractures or more had any vertebral compressions in the cohort. Similar anatomical distribution was observed as in vertebral fractures in children with glucocorticoid therapy for solid organ transplantation and in children with acute lymphoblastic leukemia (Valta et al. 2009, Siminoski et al. 2011). In the study by Siminoski, the highest rates of fractures were observed between ages 7 and 10 years.

Vertebral fracture diagnosis in children is challenging. For grading of vertebral morphology in this study, two classification methods were used (Genant et al. 1993, Mäkitie et al. 2005). The cut-off value for compression deformity was the same in both: more than 20% loss of expected height. However, whether these vertebrae in children

are low in height due to accidental crushing (loss of height), growth disturbance (no trauma), or normal variation (always been low), is not evident from a cross-sectional study, and can be discussed. Furthermore, many vertebrae for these 66 children were measured to be almost 20% lower in height than the adjacent vertebrae with normal morphology, and thus classified and reported normal while some of them may have represented true osteoporotic changes. The range of normal variability in vertebral margin deformity in growing children is large; in a study assessing vertebral body morphology in healthy children, some milder vertebral deformities, compressions of 5% to 20%, were seen in 70% of the children (Mäkitie et al. 2005). Minor changes are likely to relate to the immaturity of the skeleton in children. It is still unclear what the diagnostic criteria for asymptomatic vertebral fracture in children are. In healthy children, however, no vertebral deformity with over 20% height loss was seen. Thus, the present findings in children with frequent fractures are likely to be of importance.

Compressed vertebrae are commonly seen in advanced osteoporosis in adults, and are always a sign of pathological bone fragility; they are associated with a 5-fold increased risk of sustaining a new vertebral fracture (Black et al. 1999). In chronically ill children, the presence of osteoporotic compression fractures is increasingly recognized (Helenius et al. 2006, Valta et al. 2008, Siminoski et al. 2011, Högler et al. 2012). As these compression fractures relate to lower BMD also in children, the proper diagnosis is essential. However, the diagnosis is not clearly standardized. Symmetric short vertebral height is commonly seen in adults, and these changes, on the contrary to loss of height with end-plate changes, are not associated with lower BMD (Ferrar et al. 2007). Spinal osteochondrosis, also known as Scheuermann's disease, presents also with wedge-shaped vertebrae, mainly in the thoracic region in adolescents (Lowe et al. 2007, Masharawi et al. 2009). Plain radiographs in a cross-sectional study cannot be used to evaluate the timing of injury; further, the history of back pain in younger children is not always reliable. More longitudinal studies are needed to reveal the natural course and importance of radiographic vertebral changes in children. The anatomy of the growing spine often makes a correct diagnosis by plain radiography difficult. In addition, neither of the two classifications used in this work take into account the variety in the natural shape of vertebrae at different regions of the spine. Vertebral growth after traumatic thoracic or lumbar fracture of the spine in children occurs still at prepuberty, at least in milder compressions (Pouliquen et al. 1997). MRI could be useful in demonstrating some bony edema in fresh injuries and related disc degeneration, but is limited in availability in follow-up (Kerttula et al. 2000). In possible further research, radiographs of the spine should be taken in follow-up to demonstrate if asymptomatic vertebral compressions stay low, or recover with age, to be sure of their clinical importance and need for medication.

6.1.4 Vertebral fracture assessment

The accuracy of VFA was evaluated by comparing visibility and fracture detection rate in 65 children with spinal images obtained by densitometer and standard radiographs. The introduction of fan beam densitometers with multiple-detector array has led to faster scan acquisition and improved accuracy, both of which are important in small

subjects. Further, the radiation dose in spine imaging with densitometer is small (about 3 μS), less than the 7 μS from natural background radiation per day, and 600 μS for spine radiographs (Vokes et al. 2006), making VFA an attractive diagnostic tool in pediatrics. The visibility of vertebrae in densitometer-derived spine images seemed first not to be greatly compromised in children; only at the upper thoracic region did the summation from soft tissues limit the detection of vertebral shapes. Overall, the amount of undetectable vertebrae (1% for X-ray and 7% for VFA) was similar to that in adults (Damiano et al. 2006). However, poor visibility inhibited the readers to detect two thirds of the fractured vertebrae; half were not visible at all, and the other half misdiagnosed as of normal height and shape. Visibility and accuracy of VFA was compromised, especially in smaller and younger children, and in those with low BMD. The semi-computerized application for morphometry was not reliable to detect vertebral deformities in children in our study. Guidelines for VFA use in clinical practice have been published during the course of this work. VFA is applicable to adult population only, and the visual semi-quantitative method by Genant, used in this study, is recommended for fracture assessment in adults (Vokes et al. 2006, Lewiecki et al. 2008).

6.2. Bone health in fracture-prone children

Fractures in children are commonly thought to reflect the fact that children are naturally more active than adults; and thus, that injuries like falls are common in childhood. However, there is now increasing evidence in the literature that fractures in childhood may be related to underlying skeletal fragility. Bone health can be assessed by a variety of techniques, including non-invasive densitometry and invasive histomorphometry. In a large prospective study and meta-analysis of case-controlled studies by Clark et al., an association between low BMD and fractures in children was shown (Clark et al. 2006a, Clark et al. 2006b). The combined standardized mean difference of BMD Z-score was -0.3 between children with fractures and healthy controls. Children with fractures in these studies were commonly excluded if they were known to have some metabolic condition affecting the bone. In most of the studies, any type of fracture was counted, and all children with at least one fracture were included in “the children with fractures” group. However, fractures of the digits are more likely to result from injury-prone behavior than from reduced bone mass (Rauch et al. 2008). The present study on fracture-prone children only included children with significant fracture history defined as vertebral fracture(s) or at least two or three long-bone fractures, depending on patient’s age. Inclusion criteria used in this work were settled before the ISCD recommendations were published (Rauch et al. 2008), and are slightly more strict. History of only one lower-extremity fracture was not considered as a reason to assess bone health in the present study. We included metacarpal and metatarsal fractures, which are usually not considered as long bones in adult literature. However, for unknown reason, these fractures were not common among the present cohort of fracture-prone children. Inclusion criteria may explain the bigger difference (as in the meta-analysis) observed in BMD between the patients and controls (ranging from 0.4 for hip to 0.8 SD units for lumbar spine), despite the relatively small groups; criteria probably also explain the higher rate of forearm and

clavicle fractures in the group of fracture-prone children as compared to population-based data.

We found more subjects with hypercalciuria and hyperphosphaturia in fracture-prone children than in their age- and sex-matched controls. In a study by Olney et al., children with repeated fractures were found to have lower BMD than the non-fractured controls; and subjects with hypercalciuria accounted for all of the differences (Olney et al. 2008). Reduced BMD has been reported to be prevalent in some children with hypercalciuria (Penido et al. 2003, Freundlich et al. 2008). In line with results by Freundlich and Michalus (Michałus et al. 2008), no association between hypercalciuria and hyperphosphaturia with BMD Z-score was observed in the present study.

6.2.1 Densitometry

Densitometry is non-invasive and precise, and is applicable to children from a young age. In pediatrics, the challenge is in children's growth and interpretation of the results: body size and maturation of individuals of the same age and sex vary greatly. Among the fracture-prone children in this study, only 8% had a BMD Z-score below -2.0, thus meeting the definition of osteoporosis set by the ISCD. Similarly to adults, BMD alone has not been successful at discriminating osteoporotic patients in risk of fractures, and there is overlap in the BMD values between patients with fractures and controls. However, in all measured sites the areal BMD was, on average, lower in the fracture-prone children as compared with healthy controls. These groups did not differ in height, weight, or pubertal status. BMD by DXA is influenced by the bone size of the subject. There is also agreement on the effect of height, weight, and especially muscle mass on the skeletal parameters (Högler et al. 2003). As muscle action delivers the largest loads and strains on bone, it is likely that subjects with low muscle mass for height have a proportionately low BMC (Schoenau et al. 2002b). The present study investigated the bone in relation to lean tissue mass in fracture-prone children, and found lower BMC but no difference in bone area as compared to controls. This is in line with observations by Schoenau et al., where, in the multiple fracture group and in renal transplant recipients, the BMC to muscle cross sectional area ratio was markedly decreased, suggesting that bone strength was not adapted adequately to muscle force (Schoenau et al. 2002b). Vitamin D deficiency is related to increased osteoid, and unmineralized bone matrix could be thought to decrease the BMD measured by DXA. As vitamin D levels were equally low in patients and controls and there was no difference in height, it is likely that the observation of patients having lower BMD is valid.

The injury and possible casting of lower extremity fracture may have had an impact on the BMD in the fracture-prone children. In adults, a lower extremity fracture and the casting treatment have been shown to result in a significant loss of mineral content locally, but not at lumbar spine (Finsen et al. 1987, Petersen et al. 1997). In a study on children, a casting period longer than four weeks for tibial or femur fracture, was associated with lower BMD of the proximal femur in the injured leg (Henderson et al.

1992). However, the difference was very small (0.030 g/cm2 or 3% of the BMD of the unaffected limb) and tended to diminish with time. The higher remodeling activity and faster healing time in children may have an advantageous impact both in earlier mobilization and less amount of bone loss.

Exposure of children to ionizing radiation gives more grounds for concern than that for adults, because for children there is a longer period available for the delayed effects of radiation to manifest. Radiation doses encountered in DXA are more than two orders of magnitude less than common radiological examinations from the thorax or spine: the total effective dose from total body DXA scan is about 0.31 μSv, as compared with 50 μSv from a chest X-ray. This is negligible compared with the possible benefits from the scan (Njeh et al. 1997). Even though these doses are quite low, good radiological practice should be implemented.

Among the skeletal sites where regional DXA analyses are frequently performed (lumbar spine, hip, and forearm), only the forearm is a common fracture location in children and adolescents. Children with forearm fractures have been shown to present with lower BMD, particularly at ultradistal radius, but also at spine and total body (Goulding et al. 2005a). In some studies, BMD has been associated with future fracture risk. In the study by Flynn et al., low BMD at lumbar spine and at total body at 8 years were predictive for forearm fractures in the following 8 years (Flynn et al. 2007). However, an expert panel was unable to judge based on the available publications whether DXA measurements in children and adolescents can identify independently individuals who are at risk for developing a clinically significant fracture history (Rauch et al. 2008). Other fractures are less common in children, and it is still unclear what type of fractures should be classified as “fragility fractures” in childhood.

DXA is a method that measures bone mineral within a certain area, and most of the variance in bone strength can be attributed to bone density. Cortical bone cannot be separated from trabecular bone by DXA; neither can other bone qualities be assessed. For example, between two groups of OI with markedly different clinical phenotype, only a small difference in aBMD was observed, suggesting that factors other than aBMD are likely to influence the severity of the bone disease (Rauch et al. 2010).

6.2.2 Histomorphometry

Bone biopsy gives a direct measure of bone metabolism and remodeling. The metabolically active trabecular bone can be assessed independently of cortex, and no adjustments to skeletal size are needed in children. Many of the parameters in histomorphometry are constant with age: osteoid thickness and resorption parameters do not vary significantly. Trabecular bone volume increases due to increased trabecular thickness; and bone formation frequency and mineral apposition rate decrease continuously with age (Glorieux et al. 2000). During growth, the remodeling cycle results in a slightly higher amount of deposited bone than the amount of bone

removed; high remodeling activity is thus necessary to achieve thickening of the trabeculae in childhood.

In the present study of children with suspected primary osteoporosis, one third of the patients were found to have higher bone turnover than expected for age by histomorphometry. These children had lower BMD by DXA as compared to children with low or normal turnover; they also had more vertebral compressions. Patients with OI have low cancellous bone volume with high turnover (Rauch et al. 2000b). The diagnosis of OI is clinical, and those with known or suspected OI with typical features were initially excluded from this study. Only one patient in the present biopsy study had clinical features resembling mild disease, and his histomorphometric findings were similar to those with OI. Further follow-up and family investigations confirmed the diagnosis.

Compared to children with OI, patients with IJO have a similarly decreased cancellous bone volume but a much lower bone turnover (Rauch et al. 2000a). It appears that, in IJO, the formation defect is limited to trabecular bone and is less evident in cortical bone (Rauch et al. 2002a). It has been suggested that the impaired osteoblast function, but normal osteoclast activity, results in cancellous bone being unable to adapt to mechanical needs during growth. Thus, this would lead to fractures of metaphyses and vertebras in IJO patients in prepuberty. In the present cohort of children with suspected primary osteoporosis, the median calculated age-specific bone formation rate was -1.5 SD lower than that of normative data for children. Still, only two of the patients had findings in histomorphometry otherwise similar to IJO, and neither had clinical features typical to the disease. Although IJO is an entity described in detail already in the 1960's (Dent et al. 1965), due to its rarity, descriptions of IJO have often been limited to single case reports or to a few patients followed for a short time. Suspected patients have been reported to present with a large variety in clinical phenotypes, family history, and ages of onset (Teotia et al. 1979, Smith 1995). The term idiopathic juvenile osteoporosis has been applied to a primary osteoporosis of unknown etiology present in children, once other primary or secondary causes of osteoporosis have been considered. It has become evident that IJO is a heterogeneous group of patients with more than one etiology and mechanisms for the clinical condition. With novel techniques, some of the patients with similar clinical presentation have been found to carry mutations in the collagen genes or in the LRP5 gene (Dawson et al. 1999, Hartikka et al. 2005). Tendency to spontaneous recovery has hindered the achievement of evidence of proper treatment in IJO.

One third of the 24 pediatric patients with bone biopsy in this work were found to have low bone volume by histomorphometry. No statistical difference could be obtained for BMD Z-score by DXA between the groups of low bone volume and normal bone volume. Bone volume in histomorphometry (BV/TV) includes both mineralized and unmineralized bone volumes in a small area of trabecular compartment; DXA measures only the mineral content within the projected area, and includes both trabecular and cortical compartments. In addition, these techniques measure bone at different locations. The amount of osteoid in relation to bone volume is small even in

hypovitaminosis, median 2.6% in this study, and low vitamin D status observed in the present study patients is likely to have only a minor influence to this comparison.

Due to its invasive nature, the histomorphometry from iliac bone sample is rarely used in children. The procedure itself requires hospital resources with operating room time, and anesthesia personnel. The histomorphometric analysis is also time consuming, labor intensive, and requires special equipment and expertise. Histomorphometry of bone biopsy is expensive, has a long turnaround time on metabolic changes, and is limited to a single skeletal site (iliac bone in pelvis) due to availability with least tissue damage, and normative data. Repeated measures in follow-up are limited by the small target area. These factors have limited the use over time to special pediatric patient groups with known underlying disease. The patient group in the bone biopsy study was relatively small, although it comprised all patients available for over two years screening, and the number of subjects was almost of the same size (24 vs. 36 children) as the one used to compile normative data for this age group (Glorieux et al. 2000). It is likely that adjustment for sex, and even more for pubertal stage, would have some impact on turnover parameters for adolescent patients. Despite these shortcomings, the histomorphometry was found to be an important tool in the present study assessing primary osteoporosis in children, and the findings were not predictable by non-invasive methods.

6.2.3 Bone turnover markers

No single laboratory test is specific in the diagnosis of osteoporosis. All bone markers are dependent on bone mass and number of bone cells, as well as on remodeling activity. The biochemical markers of bone metabolism are quick to show changes in response to interventions and thus valuable in follow-up. In adults, biochemical markers of bone metabolism reflect the activity of cells in the remodeling process. Assessing single pediatric individuals in cross-sectional studies is influenced greatly by age, body size, growth velocity, and pubertal stage (Huang et al. 2011). Metabolic bone disease in children may present with altered bone turnover. In a classic example, in OI, the bone turnover is increased and the bone markers ALP and PINP can be elevated. In severe forms, however, the amount of bone is reduced, resulting in lower concentrations of markers than in controls (Schoenau et al. 2003).

In the present study, no statistical differences in bone markers ALP and ICTP between fracture-prone children and controls were found; a trend was detected for higher PINP. Bone markers are with large variability in children (Huang et al. 2011), and no follow-up studies exist to evaluate the effect of recent bone trauma on the levels. In elderly women participating in the OPRA study, the bone turnover markers were affected by a (up to 12 months) recent fracture (Ivaska et al. 2007). It remains unknown whether small differences in bone turnover markers are due to recent trauma or actual higher bone turnover in fracture-prone children. In addition, the role of new bone markers (e.g., tartrate-resistant acid phosphatase (TRACP) or osteocalcin) in clinical pediatrics remains to be elucidated.

6.2.4 Vitamin D and calcium

In this work, vitamin D levels were low in more than half of the patients, and vitamin D deficiency, defined as serum 25-OHD below 38 nmol/L, was observed in 28%. No more than one third of the fracture-prone children had been on some vitamin D substitution prior to fracture; usually they had had irregular use during the winter months. Vitamin D has been shown to be associated with the attainment of peak bone mass and to correlate positively with BMD at the radius (Outila et al. 2001, Lehtonen-Veromaa et al. 2002). In the present study, 25-OHD was positively correlated with BMD; none of the children with normal vitamin D levels had BMD Z-score below -1.0. Lower vitamin D concentrations were also associated with high turnover on histomorphometric analysis.

In osteomalacia, there is too much unmineralized bone matrix due to insufficient minerals incorporated into the matrix. In histomorphometry, osteomalacia is defined as increased osteoid thickness and increased mineralization lag time. Criteria used for adults (O.Th over 12.5 µm and Mlt over 100 d) are not applicable for children because of faster bone turnover. The cut-offs proposed for children are significantly lower (O.Th over 9 µm and Mlt over 25 d) and based on a relatively small group of apparently healthy children (Edouard et al. 2011). By these criteria, seven patients in this study's cohort had slightly increased osteoid thickness, and simultaneously increased Mlt was seen in only three. Mineralization defect can be due to insufficiency of vitamin D in blood. However, serum 25-OHD concentrations in these three patients were modest (29, 38, and 71 nmol/L), and not different from the remaining 21 patients in the study. Osteomalacia was not diagnosed in any of the subjects, but vitamin D concentrations below 50 nmol/L had an impact on osteoid parameters.

A relatively small number of reports are available on effects of vitamin D supplementation on BMD in children. A recent meta-analysis concluded that vitamin D supplementation in healthy children had limited effects; however, some although modest in those with low baseline serum 25-OHD (Winzenberg et al. 2011). Vitamin D levels are low in northern Europe, and even worse in children and adolescents living in Scandinavia (Andersen et al. 2005, Viljakainen et al. 2006). To ensure adequate vitamin D status, supplementation is needed. A 12-month supplementation with 10 µg D3 was associated with greater lumbar spine BMC augmentation in teenage girls in a study by Viljakainen et al. (Viljakainen et al. 2006). However, similar all year supplementation was not sufficient to prevent hypovitaminosis during winter months in Finland or in Canada (Lehtonen-Veromaa et al. 1999, Whiting et al. 2011). It has been calculated, that an 18 µg daily intake of D3 is required to keep serum 25-OHD within recent recommendations (above 50 nmol/L) (Cashman et al. 2011). The American Academy of Pediatrics raised US recommendations in 2008 for all infants and children to a minimum daily intake of 400 IU (10 µg) of vitamin D (Wagner et al. 2008). Even higher amounts (15 µg to 50 µg) have been recommended recently by experts in the field (Hanley et al. 2010, Holick et al. 2011). All these amounts are modest when compared with a 10–15 minute whole-body exposure to peak summer sun, generating and releasing up to 20 000 IU (500 µg) of vitamin D3 into the circulation (Hollis 2005). Sunbathers and lifeguards achieve 25-OHD levels of 250 nmol/L without evidence of

intoxication; levels of over 325 nmol have been associated with hypercalcemia (Misra et al. 2008). New Finnish national recommendations for the use of vitamin D supplements were published in 2011. However, it is questionable whether this recent recommendation of regular 7.5 µg (300 IU) daily D3 supplementation, for all children ages 2 to 18 years, is sufficient to ensure serum concentrations that are optimal for bone health in Finland, as the recommendations are designed rather to prevent extreme deficiency states.

The calcium requirements are highest during rapid growth in puberty. In the present study of fracture-prone children, the daily dairy intake was insufficient in one third of the whole cohort, and in half of the pubertal subjects. Both calcium intake and serum vitamin D concentration were lower than recommended in every fourth subject. However, no true milk avoiders without calcium supplementation were recorded. Evidence for improved BMD with calcium supplementation in healthy children and adolescents is not as clear as it is for vitamin D, and possible efficacy in fracture risk reduction remains uncertain in those who receive some calcium from diet (Winzenberg et al. 2006). Conflicting findings have also been published on the role of soft-drink consumption on reduced BMD and fracture risk. High phosphorus content, increased acid load, displacement of milk consumption by calcium-deficient soft-drinks in children's diet, or association with poor diet or unhealthy lifestyle, are all possible factors that may mediate adverse skeletal effects that have been associated with soft-drink consumption (Wyshak 2000, McGartland et al. 2003, Libuda et al. 2008). The direct effects of higher phosphate intake and FGF-23, an important regulator of renal phosphate excretion, to bone health in healthy children are yet to be clarified.

6.2.5 Life-style factors

The structural basis of bone fragility is determined before birth, takes root during growth, and gains full expression during ageing in both sexes. The peak bone mass determined by genetic factors can only be achieved with optimal life-style: several modifiable factors influence the growing skeleton. In addition to hormones, mechanical factors (physical activity and body weight) and nutrition factors (e.g., calcium, vitamin D, and proteins) are key in maximizing bone size and strength (Bonjour et al. 2009). Higher physical activity seems to be most beneficial to bone in prepuberty (Bass et al. 1998, Farr et al. 2011), but continuous and regular physical activity is valuable, even after puberty (Rautava et al. 2007). Although percentage of children reporting regular vigorous physical activity has not changed, everyday activity levels in pubertal children are decreasing (Samdal et al. 2007); this may have harmful effect on bone mass accrual. However, it is noteworthy that higher bone mass associated with increased physical activity does not seem to compensate for increased exposure to injuries, and thus number of fractures (Clark et al. 2008). In the present study, higher BMDs in patients with higher physical activity level were observed. It is possible that these children active in competitive sports form a subgroup of patients with frequent fractures without impaired bone health in general.

Being overweight was not a prevalent feature in fracture-prone children in this cohort. Obesity is considered as protective for fractures in the elderly, whereas in children excess fat mass increases the risk for fractures. Body mass is an important mechanical load challenging the skeleton, but obesity is associated with lower physical activity; increased BMI thus has a dual effect on bone. The present findings showed positive correlation between BMI and BMD measured at lumbar spine and hip; however, being overweight was not associated with better BMD in patients. Obese children with prior fracture are reported to gain bone mass poorly during adolescence, resulting in lower peak bone mass (Dimitri et al. 2010). Thus, preventive measures should be targeted to this subgroup. In this study, we found no correlation between BMI and physical activity, probably as BMI Z-score does not distinguish between fat and muscle mass.

6.3 Specific osteoporosis treatments

In adults, osteoporosis presents with enhanced bone resorption relative to formation. High-turnover osteoporosis occurs after menopause, when sudden decrease in production of estrogen weakens the bone by reducing osteoblastic activity; osteoclasts resorb bone faster than osteoblasts can function. Low-turnover osteoporosis is related to aging in adults, when osteoclasts function at their normal rate but the reduced number of osteoblasts fail to form enough new bone (Rehman et al. 1995). In both cases, antiresorptive medications can be used. Bisphosphonates are synthetic analogues of pyrophosphate, which inhibit bone resorption by inactivation of osteoclasts. In pediatric patients, the use of bisphosphonates remains controversial due to inadequate long-term efficacy and safety data; recommendations at the moment include children with recurrent extremity fractures or vertebral compressions together with reduced bone mass (Bachrach et al. 2009). In patients with OI, pamidronate treatment has resulted in a significant reshaping of flattened vertebral bodies, in decrease of fracture rate, and in increase of BMD (Glorieux et al. 1998, Rauch et al. 2004, Land et al. 2006). Defined by histomorphometry, one third of the patients in this study with suspected osteoporosis had high turnover for age, although only one had classic clinical features of OI. Equally, one third had low turnover. Only a few reports are available on bisphosphonate treatment in low-turnover osteoporosis in children (Shaw et al. 2000). Although no severe adverse effects have been reported for bisphosphonate treatment in children, the oversupression of bone modeling and remodeling is of concern during growth and fracture repair (Odvina et al. 2005). Patients with decreased osteoblast activity and normal osteoclast number/function could respond more favorably to therapeutic regimens which promote bone formation. Bisphophonates are contraindicated in patients with severe adynamic bone disease. Safety and efficacy of new medications, such as PTH and vitamin D analogues, have not yet been determined in pediatric patients (Bachrach et al. 2009). In the light of the results of the present work, routine assignment of bisphosphonates to all children with decreased BMD and peripheral fractures cannot be justified. Further research is required to establish guidelines for bisphosphonate use in pediatrics.

As histomorphometric findings in children with suspected primary osteoporosis in this study were variable, we believe that bone biopsies could be used to optimize individual treatment in the future. In fact, bisphosphonates were commenced only in one fourth of the patients with suspected severe osteoporosis after bone biopsy results. All others were ensured to have sufficient calcium and vitamin D intake for age, and clinical follow-up was organized for all.

6.4 Data collection and validation

One strength of the present epidemiological study was the prospective collection of data and patients. The Children's Hospital is the only pediatric hospital in the capital of Finland, and it reaches a large population. Children with confirmed fractures were all recorded during one whole year. The data collection was performed almost daily on all emergency patients regardless of the primary reason for seeking medical help or the primary diagnoses by the physician on call. The Finnish National Hospital Discharge Register is a reliable source for operatively treated fractures and for injuries, requiring in-hospital care, but it underestimates the incidence of minor and conservatively treated injuries. Registers, together with the unique personal number given to each individual citizen in Finland, are valuable for researchers, and have provided an opportunity for large population-based studies (Kannus et al. 1999a, Parkkari et al. 2003, Kannus et al. 2006, Helenius et al. 2009). However, most of the pediatric fractures are treated at the out-patient clinics, and in Finland not registered nationwide. Hospital registers were used to confirm inclusion of all in-patients retrospectively, and the personal numbers used to follow-up uncertain cases. Although laborious, the prospective study setting provided the option to gather detailed data for accidents and possible prior fractures in most patients.

It has been speculated that increased fracture incidence over long study period might be due to methodological factors: maybe parents are more inclined to seek medical attention for their children's injuries than they were in the 1960's, and physicians are more easily taking radiographs to confirm possible fracture diagnoses (Vitale 2010). Smaller families and urbanization are also thought to increase the use of medical facilities (Hendry et al. 2005). Thus, it was somewhat surprising to observe decreasing overall incidence of fractures from 1983 to 2005 in Helsinki. Change in the numbers of children treated entirely in private clinics may explain some of the difference; however, we believe this impact to be quite small on the total fracture incidence. Most probably including the children treated in private clinics would add to the incidence of forearm fractures due to sport injuries.

The lack of more detailed information on the trauma situations leading to fractures in children is a limitation of this work. Background data would be helpful in analyzing the causes for changed fracture pattern, and in planning prevention strategies in the future. Population-based fracture data were gathered for one urban city only, and as there is great variation in fracture incidence and pattern of fractures between countries

and environments, results of this work cannot be shifted to any other city directly. Epidemiological data should be obtained for each region separately.

Despite the large cohort for epidemiological study, the study groups of children with suspected osteoporosis retrieved from pediatric university clinic during two years were small in number. This resulted in relatively small study groups, which may have inhibited finding statistically significant differences between subgroups. In addition, some of the findings in this work are descriptive results, raising hypotheses rather than confirming any previous findings.

Study II included all consecutive patients aged 4 to 15 years with a clinically significant history of fractures. They were selected from a large cohort of children who were treated for an acute fracture during one year. Strict inclusion criteria were applied to detect apparently healthy children who were regarded as unusually prone to fractures. Those with only one long-bone fracture incident, or some underlying disease affecting the bone, were excluded; only significant fractures were counted. Thus, these results only can be applied to children with similar characteristics, not to all fractured children. However, it remains unclear as to what type of fractures should be considered as fragility fractures in children.

Patients included in the histomorphometry study (IV) comprised all the 24 consecutive patients, from Children's Hospital, who had a bone biopsy taken for suspected primary osteoporosis between March 2005 and June 2007. Those with known secondary osteoporosis or other specific diagnosis were not included. Tetracycline labeling is important for dynamic formation parameters, and it was successfully performed in all patients prior to the procedure, although in three patients, the double-label was immeasurable in trabecular bone due to impaired intestinal absorption or very low bone turnover.

Limitations of this work include the timing of assessments: biochemistry and DXA measurements for fracture-prone children were obtained by regular clinic visits, a few months after removal of the cast. Seasonal variations in vitamin D and other bone markers may have influenced some results, especially those that were compared with controls (control samples were taken only in winter-months). The effect and magnitude of fracture-induced remodeling, and those of casting/immobilization on bone markers and BMD, remain unknown and require further studies.

6.5 Future considerations

Fractures in children vary within geographical areas, but there are also commonly observed long-term changes in the fracture pattern. A national register for all fractures, not only those of hospitalized or of operatively treated patients, should be organized. With the current computerized health care system, and training of physicians for accuracy in diagnoses, a nationwide register is achievable. This would not only be a

resource for epidemiological research, but also serve for follow-up of patients and in evaluating effectiveness of treatments. The osteoporotic fractures in adults are a true economic burden in developed countries, and the majority of the costs are incurred by inpatient care (Burge et al. 2007). The rising incidence of forearm fractures and upper arm fractures in children is likely to result in a need of more extensive hospital resources in the future. Together with the reported increasing rate of operative treatment, the present findings can be used for allocations of health care resources. However, further analysis of current treatment strategies on different levels of care is needed. In addition to more unified treatment strategies, proper allocation of health care resources nationwide is of interest to policymakers. In the end, this would benefit the children with fractures with better care, most probably in more centralized pediatric trauma units.

The observed increase of incidence of forearm and upper arm fractures with simultaneous plateau or decrease of all other fracture types is of note. Both these fracture types are frequent in children, and in the present study, their increase was not related to any specific age group; associating injury situations and life-style factors deserve further studies. It may be that, as in the elderly, the impaired bone health plays a greater role in the pathogenesis of fractures at certain sites. Even more detailed fracture type evaluation in a large cohort of fracture repeaters could give some answers.

Children with significant fracture history in this study were a heterogeneous group. In some, higher fracture rate was most apparently related to higher exposure to injuries. However, in most an association with impaired bone health was observed. It would be interesting to assess all these children again as young adults, at the age of peak bone mass. Life-style factors adopted in the family, rather than inherited factors, can explain the higher prevalence of fractures in siblings in this study. Osteoporosis is a complex disease, with no single gene responsible for the higher fracture risk. Despite the genome-wide approach, current studies still struggle to find genes responsible for increased fracture risk. Although some candidate genes have been identified, hundreds of variants together with the environmental factors make this task laborious (Uitterlinden et al. 2006, Saarinen et al. 2010). Modifiable factors are likely to play a greater role; the impact of physical activity level and dietary habits on fracture frequency should be reassessed in fracture repeaters.

Direct assessment of the bone quality (including architecture, geometry, microcracs, turnover, mineralization etc.) by non-invasive methods is not yet possible in clinics. However, advances in technologies are resulting in images of higher resolution, with acceptable scanning times and radiation doses. pQCT is already used for peripheral sites; it has the advantage to separate cortical bone from trabecular bone and to measure true volumetric density. Using high-resolution pQCT (HR-pQCT), measures of three-dimensional bone geometry, overall and compartment-specific bone density, and bone microarchitecture of a specific site can be acquired within a scan time of minutes. High-resolution MRI is suitable for imaging of the trabecular network and for virtual biomechanical testing without ionizing radiation (Patsch et al. 2011). Due to

scanner geometry of HR-pQCT, it is now possible to scan peripheral sites like ultradistal radius or tibia, but not proximal parts of bones. In a recent study, correlation between peripheral HR-pQCT and histomorphometric parameters of trabecular structures for iliac bone were modest and only weakly significant (Cohen et al. 2010). In the future, non-invasive imaging methods with high resolution will partially replace the need for bone biopsy in assessment of microarchitecture, but most likely, there will remain a small group of patients with metabolic bone diseases, or otherwise abnormal turnover, who benefit from inclusion of bone histomorphometry into the diagnostic procedures (Kann et al. 2006, Tamminen et al. 2011a). In the work by Tamminen et al., children with vertebral fractures were shown to differ from other fracture-prone children without vertebral changes: despite lack of differences in conventional histomorphometry, significant changes in mineralized bone composition were observed (Tamminen et al. 2011b). Histomorphometric analysis could be further extended to evaluate possible differences in children with solely metaphyseal fractures as compared to those with diaphyseal injuries only.

7 Conclusions

The purpose of this study was to evaluate fractures in children in Helsinki, Finland. Further, children with suspected primary osteoporosis were assessed for bone health characteristics. Based on the observations in this study, the following conclusions are presented:

1. Annual incidence of overall fractures in children was 163 per 10 000 in Helsinki, year 2005; 1396 fractures in 1373 children were recorded. Fractures were almost twice as common in boys, an incidence of 201/10 000 for boys and 124/10 000 for girls was observed. Risk of fractures increases with age: the peak was seen at 14 years in boys, and at 10 years in girls. Fracture pattern has changed over recent decades (from 1983 to 2005): a decrease of overall fracture incidence by 18% and an increase in forearm fractures by 31% was observed. The rising trend for forearm fractures has been continuous over the last four decades. The decrease in fractures was most pronounced for small peripheral fractures (i.e., in hand and foot) and occurred mostly in children aged from 10 to 14 years. The increase of incidence of forearm fractures was seen in school-aged children and those younger than 7 years, both in girls and boys. The increase of forearm fractures in children has been reported to occur in many countries. Large population-based studies are scarce, but similar and simultaneous decreasing trend for other fractures has been reported earlier only for Sweden (Tiderius et al. 1999). Based on the present findings, the decrease of overall fractures and increase of forearm fractures are two parallel trends.

2. Significant fracture history, defined as a low-energy vertebral fracture or repeated fractures of the long bones, is uncommon in children. The present study found 5% of the children with acute fracture to present with such history. These children had lower mean BMD by DXA, as compared to age- and sex-matched controls. Lower calcium intake, lower physical activity, and more hyperphosphaturia were observed in patients. Fracture is recognized as a multifactorial outcome, and not all risk factors relate to bone health: patients with high physical activity were found to have normal BMD. Thus, increased fracture rate is likely to be due to risk for injuries in some, and modifiable life-style factors influence bone health in many. Both patients and controls had low vitamin D levels: more than half of the subjects had values below the recommended 50 nmol/L. Low vitamin D was associated with lower BMD, prevalent vertebral compressions, and higher turnover in bone histomorphometry.

3. Vertebral fracture was a rare diagnosis among the 1830 children with acute fracture evaluated for this work: thoracic and lumbar vertebral fractures accounted only for 1% of all fractures in the population-based cohort. Altogether, 15% of the patients with a history of long-bone fractures were found to have asymptomatic vertebral compressions in spinal assessment; compressions were associated with lower BMD and lower vitamin D concentrations.

4. Vertebral fracture assessment (VFA), spine imaging with densitometer, was evaluated in children with primary or secondary osteoporosis. The accuracy of this low-radiation method in fracture diagnosis was insufficient in small children and in those with low BMD for age, particularly in the thoracic region, due to soft-tissue summations. VFA might be applicable in older children in follow-up; however, if visibility is compromised, a standard radiograph is recommended.

5. Bone histomorphometry results were variable in children with suspected severe primary osteoporosis. One third were found to have low bone volume in biopsy; BMD determined by DXA was a poor predictor of histomorphometric results. Low BMD was related to high bone turnover, as were low vitamin D concentrations. No true osteomalacia was diagnosed in any patients, but slightly decreased values had an impact on osteoid-related parameters, thus affecting bone quality. Although invasive, bone biopsy was a useful tool in determining the need for bisphosphonate treatment.

There are no established criteria for when and how to examine children with fractures. Repeated fractures or vertebral compressions are rare findings in healthy children; this group of patients is at risk of having impaired bone health and requires thorough evaluation. Based on the present findings, these patients can be identified from the children with newly diagnosed fracture, and screening those with frequent low-energy fractures from the emergency cohort is valuable. Life-style factors, biochemical parameters, including vitamin D, and DXA should be assessed. Mineralized mass is clearly not the only factor relevant to bone strength. Attention to bone mass, as measured by DXA, however, is justified, in part because it does play a substantial role, and in part, because it is to some extent controllable. Histomorphometric findings in this work underscore the difficulties in diagnosing pediatric osteoporosis.

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