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BONE HEALTH AND VITAMIN D STATUS IN CHILDREN WITH MOTOR DISABILITY AND ADULTS WITH INTELLECTUAL DISABILITY

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ACADEMIC DISSERTATION

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by Roman numerals (I-IV):

I Kilpinen-Loisa P, Paasio T, Soiva M, Ritanen UM, Lautala P, Palmu P, Pihko H, Mäkitie O. Low bone mass in patients with motor disability: Prevalence and risk factors in 59 Finnish children. Dev Med Child Neurol. 2009 Aug 26 [Epub ahead of print].

II Kilpinen-Loisa P, Pihko H, Vesander U, Paganus A, Ritanen U, Mäkitie O. Insufficient energy and nutrient intake in children with motor disability. Acta Paediatr 2009 Aug; 98(8): 1329-33. Epub 2009 May 8.

III Kilpinen-Loisa P, Nenonen H, Pihko H, Mäkitie O. High dose vitamin D supplementation in children with cerebral palsy or neuromuscular disorder. Neuropediatrics 2007; 38(4): 167-72.

IV Kilpinen-Loisa P, Arvio M, Ilvesmäki V, Mäkitie O. Vitamin D status and optimal supplementation in institutionalized adults with intellectual disability. J Intellect Disabil Res. 2009 Dec; 53(12): 1014-23. Epub.

ABBREVIATIONS

aBMD	Areal bone mineral density
ALP	Alkaline phosphatase
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
Ca	Calcium
Crea	Creatinine
CP	Cerebral palsy
CV	Coefficient of variation
D2	Ergocalciferol
D3	Cholecalciferol
DBP	Vitamin D binding protein
DMD	Duchenne muscular dystrophy
DXA	Dual-energy x-ray absorptiometry
FNR	Finnish Nutrition Recommendations
GMFCS	Gross motor function classification system
Hb	Hemoglobin
ICD-10	International Classification of Diseases
ICTP	Carboxyterminal telopeptide of type I collagen
ID	Intellectual disability
IGF-I	Insulin-like growth factor I
IGFBP3	Insulin-like growth factor binding protein 3
IJO	Idiopathic juvenile osteoporosis
IL-1	Interleukin 1
IL-6	Interleukin 6
IM	Intramuscular
IQ	Intelligence quotient
IVA	Instant vertebral assessment
LRP5	Low-density lipoprotein receptor-related protein 5
LVA	Lateral vertebral assessment
MMC	Myelomeningocele

MRI	Magnetic resonance imaging
NIH	National Institutes of Health
NTX	N-telopeptide of type I collagen
OI	Osteogenesis imperfecta
OPG	Osteoprotegerin
P	Plasma
PBM	Peak bone mass
PEG	Percutaneous endoscopic gastrostomy tube
Pi	Phosphate
PINP	Aminoterminal propeptide of type I procollagen
PO	Peroral
pQCT	Peripheral quantitative computed tomography
Prealb	Prealbumin
PTH	Parathyroid hormone
QCT	Quantitative computed tomography
RANKL	Receptor activator of nuclear factor kappa B ligand
RDA	Recommended dietary allowance
S	Serum
SD	Standard deviation
SMA	Spinal muscular atrophy
TNF	Tumor necrosis factor
U	Urine
UVB	Ultraviolet B radiation
vBMD	Volumetric bone mineral density
VDR	Vitamin D receptor
WHO	World Health Organization
$1,25(OH)_2D$	1,25-dihydroxyvitamin D, calcitriol
25-OHD	25-hydroxyvitamin D, calcidiol

ABSTRACT

Osteoporosis is not only a disease of the elderly, but is increasingly diagnosed in chronically ill children. Children with severe motor disabilities, such as cerebral palsy (CP), have many risk factors for osteoporosis. Adults with intellectual disability (ID) are also prone to low bone mineral density (BMD) and increased fractures. This study was carried out to identify risk factors for low BMD and osteoporosis in children with severe motor disability and in adults with ID. Since vitamin D is essential for normal skeletal development, mineralization, and growth and for maintenance of skeletal health throughout life, we assessed vitamin D status and optimal means of improving status in these two patient populations.

Studies I and II included 59 children with severe motor disability, ranging in age from 5 to 16 years, who were followed at Päijät-Häme Central Hospital at the Department of Pediatric Neurology. Lumbar spine BMD was measured with dual-energy x-ray absorptiometry. BMD values were corrected for bone size by calculating bone mineral apparent density (BMAD), and for bone age when calendar age and bone age differed by more than one year. The values were transformed into Z-scores by comparison with normative data. Spinal radiographs were assessed for vertebral morphology. Blood samples were obtained for biochemical parameters of calcium homeostasis and nutrition. Parents were requested to keep a food diary for three days. The median daily energy and nutrient intakes were calculated and compared with national nutrition recommendations for age and sex.

Study III included 44 children and adolescents, aged 9 to 18 years, with severe motor disability at Ruskeasuo School, a special school for children with disabilities. After baseline blood samples, the subjects were divided into two groups; those in the treatment group received 1000 IU peroral vitamin D3 five days a week for 10 weeks, and subjects in the control group continued with their normal diet. In Study IV, 138 institutionalized adults with ID were allocated to receive either 800 IU peroral vitamin D3 daily for six months or a single intramuscular injection of 150 000 IU D3. Blood samples were obtained at baseline and after treatment.

Fractures were common; 17% of the children in Study I had sustained peripheral fractures and 25% had compression fractures. Among adults with ID, nine peripheral fractures in six patients were diagnosed during the preceding five years. BMD was low in children with motor disabilities; the median spinal BMAD Z-score was -1.0 (range -5.0 – +2.0) and the BMAD Z-score <-2.0 in 20% of the children. Low BMAD Z-score and hypercalciuria were significant risk factors for fractures. In children with motor disability, calcium intakes were sufficient, while total energy and vitamin D intakes were not. Serum concentrations of 25-OH-vitamin D (S-25-OHD) were low in all subgroups before vitamin D intervention: in almost 60% of children and in 77% of adults the S-25-OHD concentration was below 50 nmol/L, indicating vitamin D insufficiency. After vitamin D intervention, 19% of children and 42% adults who received vitamin D perorally and 12% of adults who received vitamin D intramuscularly had optimal S-25-OHD (>80 nmol/L).

This study demonstrated that low BMD and peripheral and spinal fractures are common in children with severe motor disabilities. Vitamin D status was suboptimal in the majority of children with motor disability and adults with ID and may together with inadequate weight-bearing activity contribute to poor bone health. Vitamin D insufficiency can be corrected with vitamin D supplements; the peroral dose should be at least 800 IU per day. Evaluation of bone health and prevention of osteoporosis should be included in the follow-up of children and adults with motor disability or ID.

1. INTRODUCTION

Osteoporosis is a disorder that has been considered to affect mainly the adult population, especially postmenopausal women. However, increasing evidence suggests that also children, particularly those with chronic diseases, may develop symptomatic osteoporosis (van der Sluis et al. 2001). This may be caused by the chronic disease itself or by factors related to treatment and medication of the disease. Specific risk factors consist of prolonged immobilization, malnutrition and vitamin D deficiency, use of glucocorticoids or cytotoxic agents, factors related to the underlying illness such as inflammatory cytokines, hypoxia, malabsorption, and nutritional problems, and various endocrine problems including hypogonadism and delayed growth and maturation (Sochett and Mäkitie 2005).

Children with severe motor disability have many risk factors for secondary osteoporosis. Most importantly, their weight-bearing activity, which is known to be crucial for normal bone mass accrual, is reduced. Many children with severe motor disability also have feeding problems and suboptimal calcium, vitamin D and other nutrient intakes because of oral-motor difficulties, dysphagia, vomiting, and constipation (Sullivan et al. 2000). People with severe motor disabilities are often housebound and have reduced sunlight exposure, and consequently, reduced serum vitamin D concentrations. Furthermore, recent studies have shown that antiepileptic drugs, commonly prescribed for children with severe motor disabilities, may cause bone loss (Fitzpatrick 2004). Low bone mass and bone mineral density (BMD) have been observed in a significant proportion of children with cerebral palsy (CP) (Henderson et al. 2002).

Similar risk factors for impaired bone health are present also in some adults with intellectual disability (ID) if their moving ability is reduced, especially if they are institutionalized. People with ID often have reduced mobility and outdoor activities, use of antiepileptics, poor nutrition, and vitamin D insufficiency (Jaffe et al. 2005). Other risk factors include hypogonadism or therapeutic amenorrhea (Arvio et al. 2009). Many of these factors may be present from early childhood, influencing the acquisition of bone mass. Low BMD and osteoporosis are common in adults with ID (Aspray et al.

1998). Further, fractures are 1.7-3.5 times more frequent in adults with ID than in the normal population (Tannenbaum et al. 1989, Lohiya et al. 1999).

Vitamin D deficiency is a worldwide problem and is associated with various health outcomes (Holick 2004). Low vitamin D concentration results in a negative calcium balance and in secondary hyperparathyroidism, both of which have a negative impact on bone mineral content (BMC) and BMD. Vitamin D is synthesized in the skin with ultraviolet radiation from the sun or absorbed from food in the gut (Holick 2004). In Finland, dairy products are fortified with vitamin D. However, some studies have shown that vitamin D intakes in the general Finnish population remain inadequate (Lamberg-Allardt et al. 2006) to compensate for the lack of sunlight exposure, especially in the wintertime. Adequate vitamin D intake and serum levels are particularly important in people with motor disabilities, who already are at an increased risk of developing symptomatic osteoporosis because of decreased weight-bearing capacity.

Bone health and vitamin D status in children or adults with severe motor or intellectual disabilities have not been previously studied in Finland. We therefore carried out a study to assess skeletal health and associated risk factors in Finnish patients and to evaluate different vitamin D doses to determine optimal vitamin D supplementation in these patient groups.

2. REVIEW OF THE LITERATURE

2.1. Bone structure

The skeleton has multiple functions. It provides mechanical support for weight bearing and moving and protects internal organs, but also stores vast quantities of calcium, phosphate, and magnesium, most of the calcium and phosphate of the body being stored in the skeleton (Baron 2003). Further, bone marrow serves as a place for hematopoiesis (Baron 2003). Two types of bones can be distinguished anatomically: flat bones (skull, scapula, mandible, ileum) and long bones (e.g. tibia, femur, and humerus).

Bone tissue is macroscopically divided into cortical (compact) and trabecular (cancellous, spongy) bone. Cortical bone is found in the diaphyses of long bones, while trabecular bone is present at the ends (metaphyses) of long bones, in vertebrae and nearby joint surfaces, and in flat bones (Baron 2003). Approximately 80% of the skeleton is cortical bone and 20% trabecular bone. These bone types differ in metabolic activity, trabecular bone being more active and involved in mineral homeostasis. Twenty-five percent of trabecular bone, but only 2-5% of cortical bone, is replaced every year (Baron 2003).

Bone is composed of mineral (50-70%) and organic components (20-40%), water, and lipids. The major mineral component in bone extracellular matrix is hydroxyapatite (95%), and the remainder consists of calcium carbonate, calcium citrate, and magnesium. The organic bone matrix is mainly (90%) composed of type I collagen fibers. The remaining matrix is formed by noncollagenous proteins including, glycoproteins, osteocalcin, sialoproteins, and proteoclygans. Bone cells, i.e. osteoblasts, osteoclasts, and osteocytes, comprise only 2% of the organic bone component. (Baron 2003).

2.2. Bone cells and bone metabolism

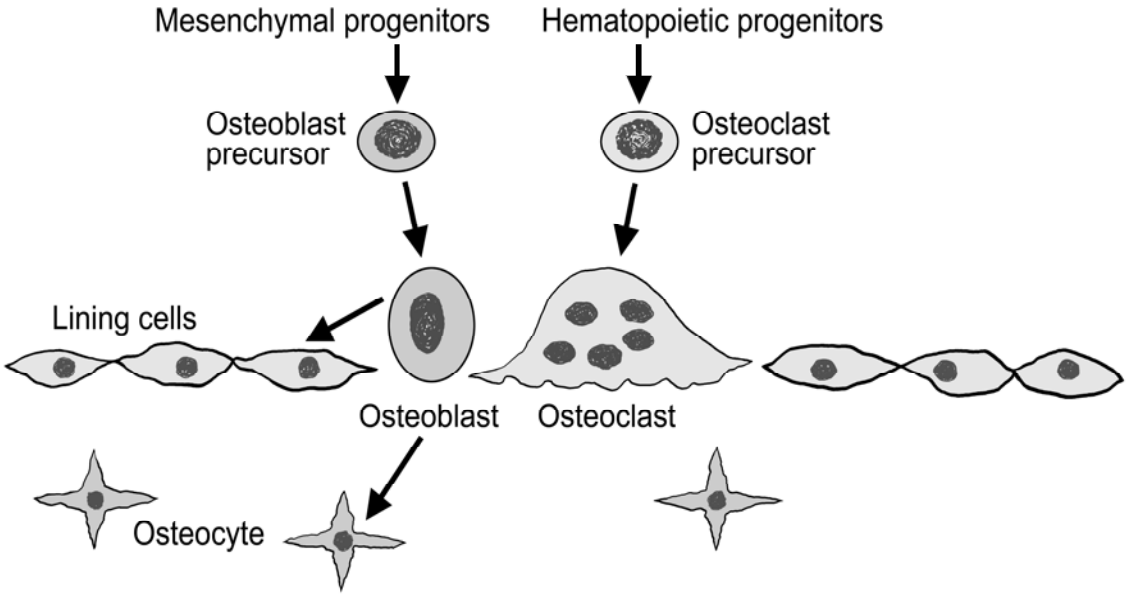

Figure 1. Origin of osteoblasts, osteocytes, and osteoclasts. Osteoblasts originate from mesenchymal stem cells and mature into osteocytes. Osteoclasts originate from hematopoietic stem cells.

Osteoblasts

Osteoblasts arise from multipotent precursor cells of mesenchymal origin (Figure 1). Osteoblasts are responsible for bone formation and secrete collagen and other bone matrix proteins. The organic bone matrix is called the osteoid. Osteoblasts also contribute to mineralization of the osteoid by secreting several proteins, such as alkaline phophatase, osteocalcin, and osteopontin, that are essential in the mineralization process. Osteoblast activity is regulated by numerous hormones and cytokines. Osteoblast lineage cells regulate osteoclasts by secreting osteoprotegerin (OPG), which is an important inhibitor of osteoclastogenesis. Osteoblast lineage cells have receptors for e.g. 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] and parathyroid hormone (PTH). (Baron 2003, Grabowski 2009).

Osteoclasts

Osteoclasts originate from the hematopoietic lineage (Figure 1) and are responsible for bone resorption. Osteoclasts function on the surface of calcified bone. The key regulators of osteoclastogenesis are receptor activator of nuclear factor kappa B ligand (RANKL) and OPG. RANKL and OPG are produced by osteoblasts. Mature osteoclasts secrete acid and proteolytic enzymes causing bone resorption. Degradation products, including collagen fragments, phosphate, and calcium, are released into the circulation. In an optimal/normal situation, bone formation and resorption are coupled. (Grabowski 2009).

Osteocytes

Osteocytes originate from osteoblasts (Figure 1). Osteocytes comprise the largest proportion of cells in mineralized bone and give support to bone structure by forming numerous cytoplasmic connections with adjacent cells. Remodeling is thought to be mediated by osteocytes. Further, osteocytes respond to mechanical strain on bones and mediate signals for bone formation and resorption. Osteocytes may undergo apoptosis, which is important for skeletal development, but also contributes to bone loss in osteoporosis. (Lian et al. 2003).

Bone modeling and remodeling

During growth the bones maintain their normal shape by bone modeling (Baron 2003). The process of bone maintenance, in which old bone is removed and then replaced by new bone, is called remodeling (Figure 2). Modeling and remodeling mechanisms are influenced by a vast variety of systemic and local factors, and they respond rapidly to the body’s metabolic homeostasis. Bone formation is mediated by osteoblasts and bone resorption by osteoclasts (Baron 2003).

Bone is continuously turned over in the remodeling process, which consists of bone resorption and formation in a lifelong process with successive cycles (Mundy et al. 2003). In this process, osteoclasts resorb old bone tissue. This part is soon replaced by

new bone made by osteoblasts. Remodeling maintains the normal shape of bones and renews bone tissue. Further, through remodeling, damaged bone tissue can be removed and replaced by new bone. Normally, the activities of osteoblasts and osteoclasts are balanced in adults, and remodeling has no effect on the amount of bone. Bone loss occurs, as in menopausal osteoporosis, when the amount of bone resorption is higher than the amount of bone formation. Trabecular bone is more active in remodeling than cortical bone (Mundy et al. 2003).

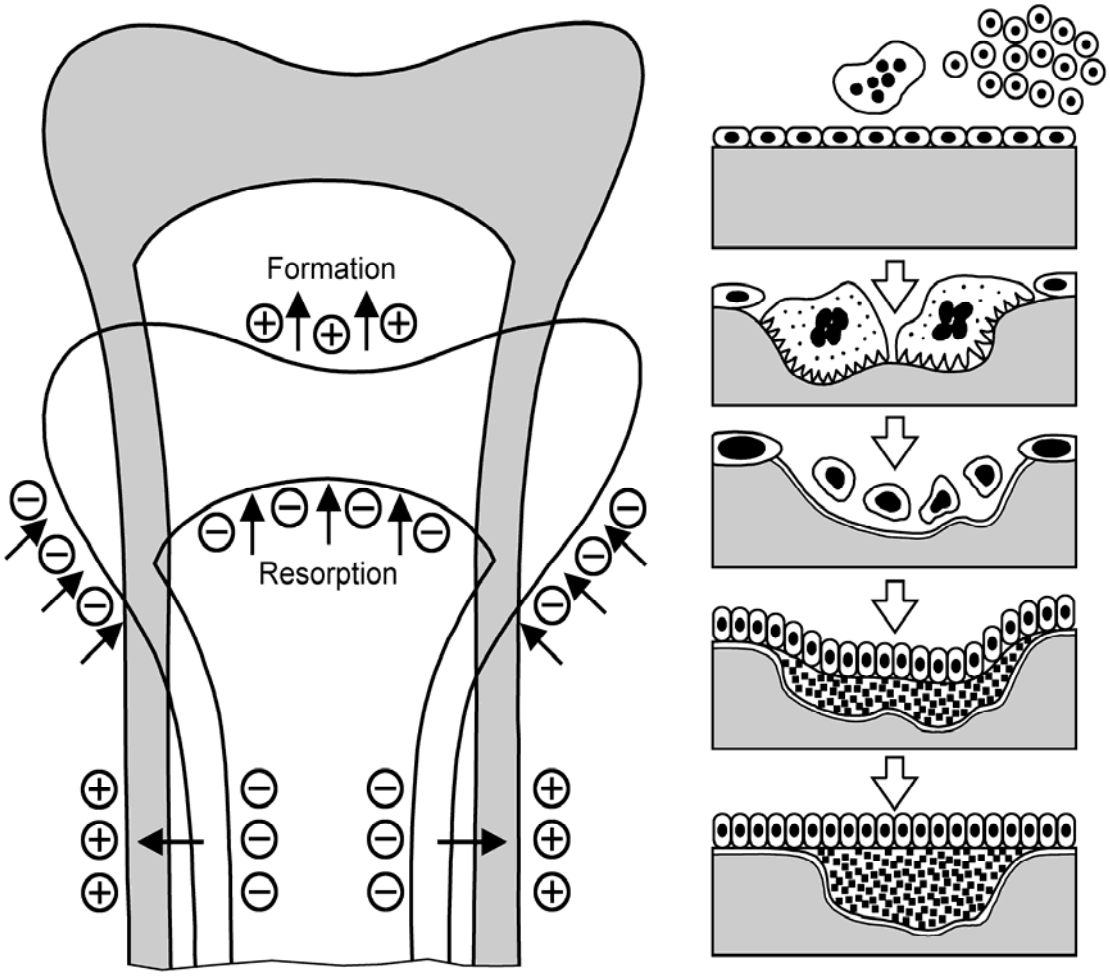

Figure 2. Bone modeling and remodeling. Osteoclasts break down old bone tissue. This is soon followed by osteoblast activity; osteoblasts form new collagen and other matrix proteins. The collagenous matrix then undergoes mineralization (Adapted from Baron 2003).

In the modeling process, osteoblasts form new bone without prior bone resorption, and consequently, an increase in the amount of bone tissue is possible (Mundy et al. 2003). Further, osteoblasts form more bone than osteoclasts remove, and this leads to net increase in bone tissue. During childhood there is more modeling than remodeling. Modeling maintains the normal shape of bones during growth and is responsible for the increase in bone circumference during growth (Mundy et al. 2003).

Both bone modeling and remodeling are influenced by parathyroid hormone (PTH), sex, growth, and thyroid hormones; glucocorticoids; growth factors, cytokines, and prostaglandins; hereditary and nutritional factors; and physical activity (Mundy et al. 2003, Seeman and Delmas 2006, Shapiro 2008).

Longitudinal bone growth is called endochondral bone formation and occurs mainly in growth plate cartilages (Karsenty and Kronenberg 2002). Growth plates are present in the ends of long bones. In the growth plates, mesenchymal cells condense and turn into chondrocytes. Chondrocytes synthesize collagens and other matrix molecules. This spongiosa is subsequently remodeled into mature trabecular bone.

2.3. Bone mass accrual

Bone mass gain is accelerated between 11 and 13 years in girls and 13 and 17 years in boys, coinciding with puberty. In adolescent females, bone mass gain declines rapidly after menarche, while in males it remains significant up to the age of 20 years (Bonjour et al. 1991). Bone mass (and strength) achieved by the end of growth period is known as peak bone mass (PBM) (Figure 3). Especially during the years of high bone mass gain, bones are vulnerable to effects of chronic conditions (Bonjour et al. 1991, Kröger et al. 1993).

Bone mass gain is similar in boys and girls in prepuberty. Sex steroids regulate bone growth in puberty. In boys, the onset of puberty occurs later than in girls and the period of increased bone growth lasts longer, which is thought to be the reason for the gender difference in PBM after puberty (Theintz et al. 1992). In puberty, boys gain more bone mass as a result of the greater bone size increment induced by testosterone. At the end

of puberty, cortical thickness is greater in males. Estrogen, growth hormone, and insulin-like growth factor I are responsible for pubertal growth and attainment of PBM in girls (Bonjour et al. 2009). Estrogens are essential for longitudinal bone growth, and they accelerate bone growth at the beginning of puberty. Estrogens are also needed for closure of growth plates in both sexes (Bonjour et al. 1994, Seeman 2002).

Bone mass begins to diminish after the age of 40 years. In women, this reduction is faster due to the decline in estrogen concentration in menopause. PBM is a strong determinant of bone health in adulthood; the greater the achieved PBM, the smaller the risk for osteoporosis and fractures later in life (Bonjour et al. 1991, 2009). An increase in PBM by one standard deviation is estimated to reduce the fracture risk by 50% (Bonjour et al. 2009).

Several factors, including physical activity, nutrition, race, gender, hormonal status, and pubertal developmental stage, chronic illnesses, and medication, can affect PBM accretion during childhood and adolescence, although 60-80% is determined by genetic factors (Slemenda et al. 1991).

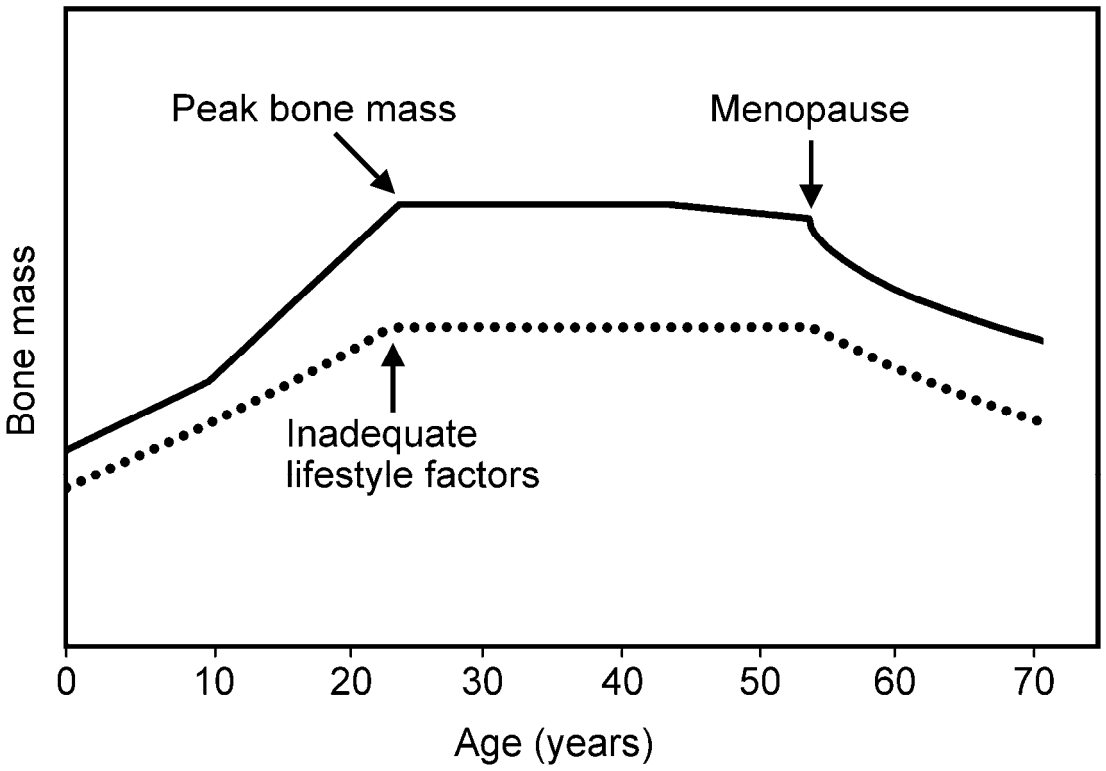

Figure 3. Peak bone mass is attained by 20 years of age. Several factors, including genetic factors, lifestyle factors, and hormones, can affect the magnitude of peak bone mass.

Effect of physical activity on bone

Physical activity during childhood and adolescence appears to be an important predictor of bone mass. Children who are physically more active and participate in sports have a higher BMD than those who are less active (Cassell et al. 1996, Janz et al. 2001). It is not clear whether the positive effect of physical activity in youth is extended to older age and results in reduced fracture risk. However, recent studies have shown that women who were physically more active in adolescence preserved higher BMC later in life, even after menopause (Baxter-Jones et al. 2008, Kato et al. 2009). In children with chronic diseases, especially these with motor disabilities, physical activity can be impaired, affecting their BMD.

The so-called mechanostat and muscle-bone-unit theories attempt to explain the effect of physical activity and mechanical forces on bone strength (Frost and Schönau 2000). These theories suggest that control of bone strength is dependent upon muscle load on bone, and bone mass and strength are related to muscle function. The theories further suggest that mechanical loads, which are muscle functions, result in bone strains, causing osteoblasts to stimulate bone modeling and leading to increased bone strength (Frost and Schönau 2000). If strains to bones are low, remodeling removes bone on the endocortical surface, resulting in a thinner bone cortex. Maximal force activities with intense short spurts of repetitive muscular contractions increase bone strength more than low-force static activities (Schönau 2005).

2.4. Assessment of bone health

Different means of evaluating bone metabolism, bone mass, and bone strength exist. Most of these methods have been developed mainly to study postmenopausal osteoporosis, and their use in the evaluation of the growing skeleton is not as well established.

Dual-energy x-ray absorptiometry

Dual energy x-ray absorptiometry (DXA) has been the gold standard for BMD assessment in adults since the late 1980s, and more recently, it has also been widely used in children (Kanis 2000, Crabtree and Ward 2009). DXA is a noninvasive method to quantify bone mass; total body composition and fat content can also be measured. DXA measures BMC as an attenuation of the x-ray by the scanned bones. The result is given as a T-score, which signifies the number of standard deviation (SD) units that a BMD measurement differs from the mean of sex-matched young adult reference data (Kanis 2000). Z-scores instead of T-scores should be used in children (Bishop et al. 2008). A Z-score compares measurements with age-, and gender-specific reference data. In children, bone size and maturation should be considered by using bone age instead of calendar age (Fewtrell 2003, Bishop et al. 2008, Crabtree and Ward 2009).

The radiation dose of DXA measurement is dependent on measurement site, but is usually small (0.1-6 μSV) compared with many other radiological assessments (Säteilyturvakeskus 2009). This is important in children, who are sensitive to radiation. Further, the imaging time is short, allowing assessment of bone mass also in young children with poor cooperation. DXA measures areal, not volumetric BMD; BMC (g) is divided by the area (cm^2) of the scanned site (Carter et al. 1992, Crabtree and Ward 2009). It provides a BMD expressed as grams per area of anatomic site (g/cm^2). DXA does not measure true BMD, which is bone mass divided by bone volume (g/cm^3). Mathematical models have been created to correct for bone size and calculate bone mineral apparent density (BMAD) (Kröger et al. 1993).

Because of the two-dimensional nature of the technique, DXA may underestimate BMD in children with short stature (Fewtrell et al. 2005). DXA does not take into account the depth of bones. Thus, DXA is size-dependent and has limitations in measuring children in whom bones change in size and shape during growth. Furthermore, especially in children with chronic illness, pubertal maturation should be considered by using bone age instead of calendar age (Fewtrell et al. 2005, Valta et al. 2008, Crabtree and Ward 2009).

According to a recent statement of the International Society for Clinical Densitometry Official Positions, skeletal sites recommended for DXA assessment in children are the lumbar spine (posterior-anterior) and the total body excluding the head (Gordon et al. 2008). The proximal femur, unlike in adults, is not considered a reliable measurement site in growing bone due to significant variability in the skeletal maturation of this site. According to the Positions, DXA is the only method recommended for pediatric use in the assessment of bone mass in clinical settings (Gordon et al. 2008).

Quantitative computed tomography

The main limitation of DXA is that it uses information gained from a two-dimensional projection to assess a three-dimensional structure. By contrast, quantitative computed tomography (QCT) provides a three-dimensional image, which enables the measurement of true volumetric density (vBMD), and thus provides size-independent measures of trabecular and cortical bone (Specker and Schoenau 2005). The fact that this method has a 10- to 12-fold greater dose for ionizing radiation than DXA has led to the use of peripheral quantitative computed tomography (pQCT). The radiation dose, approximately < 1 μSV, is much lower than in normal QCT (Specker and Schoenau 2005). The sites of measurement are the radius, tibia, and femur. Pediatric pQCT reference data are available for the radius (Rauch and Schönau 2008).

Quantitative Ultrasound

Quantitative ultrasound was developed in the 1980s. It is a nonionizing and noninvasive method, and the equipment is portable (Specker and Schönau 2005). The measurement is based on the attenuation of the ultrasound beam when it passes through bone. Calcaneal and phalangeal reference values are available for both adults and children.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) enables trabecular bone to be distinguished from cortical bone (Crabtree and Ward 2009). MRI provides a volumetric measure, and bone

strength and trabecular architecture can be calculated. MRI is used mainly for research purposes, and its use in clinical practice is limited (Crabtree and Ward 2009).

Radiography

Evaluation of BMD from conventional radiographs is not accurate; BMD can be reduced by more than 30% until it becomes visible in a radiograph. Instead, conventional radiographs can be used to assess vertebral morphology and osteoporotic fractures (Genant et al. 1993).

A pediatric scoring method for evaluation of vertebral changes has also been developed (Mäkitie et al. 2005). This method was created for the evaluation of changes in vertebral morphology in childhood secondary osteoporosis. Morphological changes are classified as normal or mild deformities (grade 0, 1a, 1b, 1c) when the compression is less than 20%.The changes are considered abnormal if the anterior, middle and/or posterior part of the vertebra is compressed by 20% or more (2a, 2b, 3a, 3b). These changes can further be classified as mild anterior wedge deformities, 2a, when there is 20-49% anterior height reduction, as severe anterior wedge deformities, 2b, when the anterior height reduction is ≥50%, as mild compression deformities, 3a, when there is 20-49% middle height reduction, or as severe compression deformities, 3b, when there is ≥50% middle height reduction (Figure 4) (Mäkitie et al. 2005).

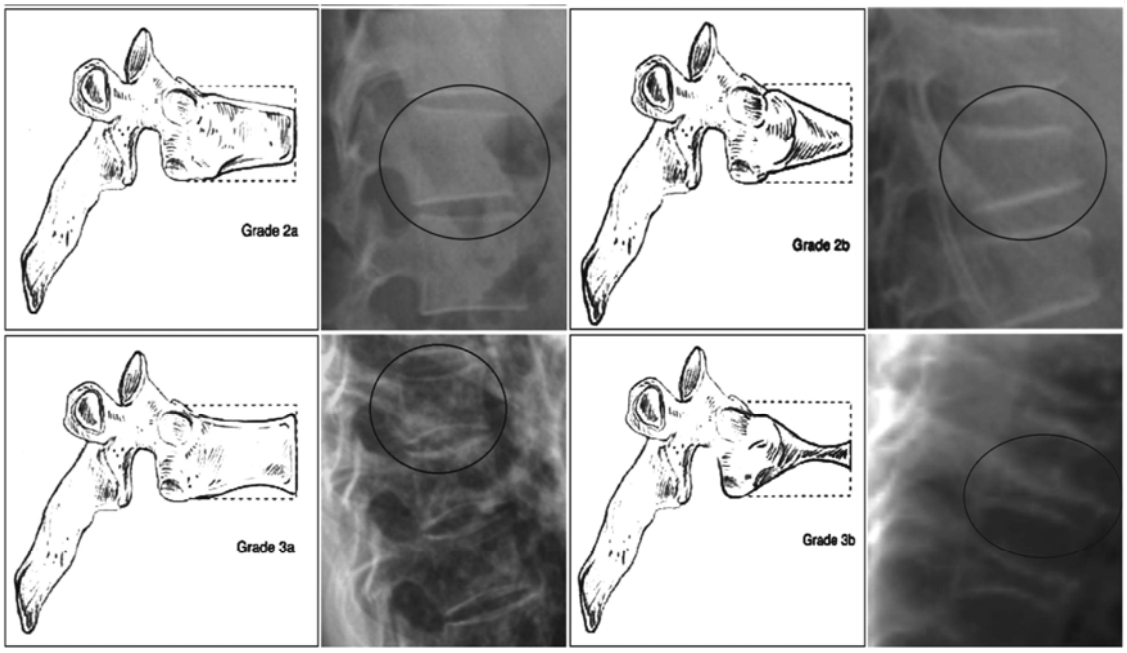

Figure 4. Pediatric grading method for vertebral compression fractures in secondary osteoporosis. Reprinted from Mäkitie et al. 2005 with the permission of Elsevier Ltd.

Spinal imaging with DXA can also be used to assess vertebral morphology. Instant vertebral assessment (IVA, Hologic densitometers) (Mäyränpää et al. 2007) and lateral vertebral assessment (LVA, Lunar densitometers) (Binkley et al. 2005) may not be as accurate as conventional radiographs, but the radiation dose is much lower than with radiography; this method thus has its advantages for use in children.

Bone biopsy

The best method to assess bone structure and metabolism is an invasive bone biopsy (Rauch 2009). Biopsy is usually obtained from the iliac crest with a needle. A good sample contains two cortices separated by a trabecular compartment. Biopsy samples can be assessed qualitatively and quantitatively; trabecular and cortical bone structure,

the mineralization process, the activity of bone metabolism, and bone cells can be examined (Rauch 2009). Quantitative analysis by computerized histomorphometry is an important method to examine bone metabolism; the analysis focuses on remodeling of trabecular bone. A bone biopsy is indicated if the diagnosis is unclear with noninvasive methods in a patient with vertebral compression fractures or multiple long bone fractures caused by minor trauma.

Bone markers

Biochemical markers of bone turnover, or bone markers, have proven useful and noninvasive tools for studying bone metabolism (Seibel 2005, 2006). Their main use is in monitoring response to bisphosphonate treatment. These biochemical markers can be assessed in urine or serum. Markers for bone resorption include collagen degradation products, carboxyterminal telopeptide of type I collagen in serum (ICTP), N-telopeptide of type I collagen in serum and urine (NTX), noncollagenous proteins of the bone matrix (osteocalcin), and enzymes expressed and secreted by osteoclasts during bone resorption (Seibel 2005, 2006). Markers of bone formation are products of osteoblastic activity, which can be measured in serum. These include alkaline phophatase (ALP) and aminoterminal propeptide of type I collagen (PINP) (Seibel 2005, 2006). In adults, bone resorption markers are more useful in prediction of fractures than bone formation markers. The use of bone markers in pediatrics is not well established (Rauchenzauner et al. 2007).

2.5. Osteoporosis

Definition and prevalence of osteoporosis

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue, which lead to an increase in bone fragility and susceptibility to fracture (National Institutes of Health [NIH] 2001). In adults, the diagnosis of osteoporosis is usually based on BMD measurements by DXA. The preferred measurement sites are the lumbar spine from L1 or L2 to L4, the femoral neck, and total hip (Kanis and Glüer 2001). In adults, the measured BMD value is compared with the average of young healthy individuals and expressed as a T-score. In adults, osteoporosis is defined by the World Health Organization as BMD less than -2.5 SD below the normal mean (T-score < -2.5), as measured by DXA (WHO 2003). Osteopenia is defined as a T-score between -1.0 and -2.5.

Osteoporosis is a major public health concern. An estimated 30 000 – 40 000 osteoporotic fractures occur in Finland every year (Käypä hoito -suositus 2000), and these numbers are increasing with people getting older. The most serious consequence of osteoporosis is hip fracture, which is associated with a 20-30% mortality. Another 30% of patients with hip fracture have permanent deterioration of independent functional capacity. At the beginning of the century, the annual cost for a single hip fracture was approximately 15 000 € (Nurmi et al. 2003). If a fracture led to permanent institutional care, the average cost increased by 2-3 fold.

Definition of osteoporosis in children

According to a recent statement of the International Society for Clinical Densitometry, in children and adolescents, the diagnosis of osteoporosis requires both the presence of a significant fracture history and low BMC or BMD (Rauch et al. 2008). Low BMC or BMD is defined as a Z-score less than or equal to -2.0, with the Z-score adjusted for age, gender, and body size of the child. Fracture history is considered significant if the child or adolescent has one or more of the following: 1) a long bone fracture of the

lower extremities, 2) vertebral compression fractures, or 3) two or more long-bone fractures of the upper extremities (Rauch et al. 2008).

The 2000 NIH Consensus Development Conference on Osteoporosis Prevention, Diagnosis, and Therapy identified bone mineral accretion during childhood as a critical determinant of osteoporosis later in life (NIH 2001). Chronic diseases, e.g. inflammatory diseases and hormonal disturbances, impaired physical activity, nutritional problems, and medication used in these conditions may have a negative effect on bone mineral accretion during childhood, and normal PBM may not be achieved (van der Sluis and de Muinck Keizer-Schrama 2001, Leonard and Zemel 2002, Sochett and Mäkitie 2005).

Primary osteoporosis

Osteoporosis can be divided into primary and secondary osteoporosis. In adults, primary it can further be subclassified into three types (Glaser and Kaplan 1997). First, classical osteoporosis is found in postmenopausal women and is due to decreased estrogen levels. Primary osteoporosis is also common in both elderly men and women and is due to aging. In idiopathic form, no reason for bone loss is apparent.

In childhood, osteogenesis imperfecta (OI) is the most common cause of primary osteoporosis. OI is a rare disease and can be divided to eight subtypes varying in severity (Rauch and Glorieux 2004). Most types are autosomal dominant and result from abnormalities in type I collagen synthesis or processing. The most severe forms cause perinatal death, while in mildest forms symptoms may be scarce. The diagnosis is based on clinical examination. Genetic testing, DXA, and bone biopsy may be helpful in setting a diagnosis (Bishop 2009).

Idiopathic juvenile osteoporosis (IJO) is a rare condition in which a previously healthy prepubertal child presents with vertebral and long bone fractures (Mughal 2009). The child may have difficulty in walking due to proximal muscle weakness and chronic pain. The etiology of IJO is likely to be multifactorial, and several genes may contribute. Osteoporosis-pseudoglioma syndrome is an autosomal recessive inherited

condition characterized by low bone mass, fragility fractures, and blindness (Mughal 2009). The condition is caused by homozygous loss-of-function mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) gene (Gong et al. 2001). In a recent study by Hartikka et al. (2005), heterozygous mutations in *LRP5* were associated with primary osteoporosis in children.

Secondary osteoporosis

Secondary osteoporosis can be found in both adults and children. In secondary osteoporosis, bone loss is due to an illness, medications, or lifestyle factors. Secondary osteoporosis is more common in young adults and children than primary osteoporosis. Increasing evidence suggests that reduced bone mass is associated with increased fracture risk and osteoporosis not only in adults but also in chronically ill children (Clark et al. 2006). Causes and mechanisms of secondary osteoporosis in children are presented in Table 1. In disease, the normal balance of bone formation/bone resorption is disturbed, causing alterations in bone mass accrual.

Table 1. Causes of secondary osteoporosis in childhood. IL-1, interleukin 1; IL-6, interleukin 6; TNF, tumor necrosis factor. * *Mechanisms are disorderspecific.*

Condition	Disorders	Mechanism of osteoporosis
Inflammation	Juvenile idiopathic arthritis Inflammatory bowel disease Crohn's disease Cystic fibrosis Systemic lupus erythematosis Dermatomyositis Juvenile idiopathic arthritis	Glucocorticoids Inflammatory cytokines (IL-1, IL-6, IL-11, TNF)
Inadequate physical activity	Cerebral palsy Neuromuscular disorders Spina bifida Head/spinal cord injury	Lack of weight bearing Decreased muscle mass
Endocrine disorders	Turner syndrome Klinefelter syndrome Hypopituitarism Anorexia nervosa Hyperthyroidism Hyperparathyroidism Diabetes mellitus	**Estrogen deficiency* *Testosterone deficiency* *Growth hormone deficiency* *Estrogen deficiency, malnutrition* *Thyroxin excess* *Parathyroid hormone excess*
Medications	Glucocorticoids Anticonvulsants Anticoagulants Cyclosporine A	Impaired osteoblast and osteoclast function Abnormal vitamin D metabolism
Nutritional deficiencies	Malnutrition Inadequate intake of calcium Malabsorption Celiac disease Lactose intolerance Anorexia nervosa	Inadequate vitamin D and calcium intake
Renal disorders	Chronic renal failure Nephrotic syndromes	Hyperparathryroidism
Hematological conditions	Thalassemia Hemophilia Sickle cell disease	
Others	Organ transplantation Tumors	Underlying disease, glucorticoids Chemotherapy, irradiation

2.6. Severe motor disability

Severe motor disability is common in children treated in pediatric neurology units. CP occurs approximately in 2/1000 live births (Surveillance of Cerebral Palsy in Europe 2002). Motor disability of CP patients is often accompanied by disturbances of sensation, perception, cognition, communication, and behavior, and by epilepsy (Rosenbaum et al. 2007). One-fifth of children with CP have severe intellectual deficit and are unable to walk (Surveillance of Cerebral Palsy 2002). Other diseases resulting in motor disability in childhood include e.g. neural tube defects, neuromuscular disorders, and various chromosomal anomalies leading to intellectual impairment and inability to move. Also brain and spinal cord injuries cause severe motor disability in childhood. According to a recent study from Switzerland, the prevalence of neural tube defects in liveborn children was 0.13/1000 (Poretti et al. 2008). The major group (70%) of these defects consisted of myelomeningocele (MMC). According to the Finnish Register of Congenital Malformations, the prevalence of neural tube defects during 1993-2006 in Finland was 7.4/10 000 births (available at www.stakes.fi). Recent estimates for the prevalence of genetic muscle diseases in the British population was 8.46/100 000 for dystrophinopathies and 1.87/100 000 for spinal muscular atrophy (SMA) (Norwood et al. 2009).

Classification of gross motor deficits

The Gross Motor Function Classification System (GMFCS) was developed to standardize and classify the level of motor disability and functional limitations in children with CP (Palissano et al. 1997). It is a five-level grading scale (Table 2) in which emphasis is on self-initiated movement during sitting, standing, and walking. GMFCS was initially developed for children with CP from 2 to 12 years. It was recently extended to adolescents (Palissano et al. 2008).

Table 2. Gross Motor Function Classification System (GMFCS).

GMFCS	
Level I	The child can walk without restrictions, but has limitations in more advanced motor skills.
Level II	The child is able to walk without assistive devices, but has limitations in walking outdoors and in the community.
Level III	The child walks with assistive mobility devices and has limitations in walking outdoors and in the community.
Level IV	The child is transported and self-mobility is limited. Power mobility is used outdoors and in the community.
Level V	Self-mobility is severely limited, even with the use of assistive technology.

2.7. Intellectual disability

ID is caused by a disease, disorder, or damage affecting the brain. In the International Classification of Diseases (ICD-10) (WHO 2007), it is defined as a condition of arrested or incomplete development of the mind. ID is characterized by impairment of cognitive, language, motor, and social abilities, and manifests before the age of 18 years. The intelligence quotient (IQ) criterion for ID is <70 with a standardized intelligence test. ID is considered mild if IQ is from 50 to 69; moderate if IQ is from 35 to 49; severe if IQ is from 20 to 34; and profound if IQ is less than 20. The etiological factors for ID can be classified in many ways, but in general the factors can be divided into 1) genetic disorders, 2) central nervous system malformations, 3) external prenatal factors, 4) perinatal disorders, 5) postnatal disorders, and 6) disorders of unknown etiology (Wilska and Kaski 1999). ID is frequently associated with other disorders such as CP or other motor disability, visual or auditory impairment, impaired speech, eating and swallowing difficulties, and epilepsy (Arvio and Sillanpää 2003). The prevalence of ID in Finland in recent studies has been 0.7- 1.1% (Heikura et al. 2003, Westerinen et al. 2007).

2.8. Bone health in children with severe motor disability

In children with severe motor disabilities, reduced physical activity and decreased muscle mass and strength are obvious risk factors for secondary osteoporosis. Many of these children also have oral-motor difficulties and dysphagia, predisposing to feeding problems and suboptimal energy, calcium, and vitamin D intakes (Sullivan et al. 2000). The use of antiepileptic drugs is an additional risk factor for bone loss (Fitzpatrick 2004).

Some previous studies on bone quality and risk factors for osteoporosis and fractures in children with severe motor disability exist (Table 3). According to these earlier studies, low bone mass and fractures are frequent in children with motor disabilities. However, the results are not consistent, and the factors contributing to impaired bone health and the role of vitamin D in pathogenesis remain largely unknown.

Table 3, next page. Findings of some earlier studies in children with severe motor disability. BMD, bone mineral density; CP, cerebral palsy; GMFCS, gross motor function classification system; MMC, myelomeningocele; NA, not available; S-25-OHD, serum 25-hydroxyvitamin D; U-Ca urine calcium.

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Inclusion criteria	Reference	N	Mean age (range)	BMD (mean Z-score)	Fractures peripheral/ vertebral	S-25-OHD (nmol/L)	Risk factors for skeletal fragility
CP							
Quadriplegia	Henderson 1997	43	7.9	Spine -1.5 Femur -2.8	35% / NA	Mean 53	Previous fracture
GMFCS≥3	Henderson 2002	117	9.7	Femur <-2.0 in 77%	26% / NA	<50 in 53%	High GMFCS Low skin-fold thickness Feeding difficulty Anticonvulsants
GMFCS≥3	Henderson 2004	107	10.9	NA	31% / NA	NA	Low weight Z-score Prior fracture Anticonvulsants Feeding difficulty Age
Quadriplegia	King 2003	48	15	Spine -2.4	39% / NA	Mean 74	NA
GMFCS≥3	Henderson 2005	69	NA	Longitudinal changes	NA / NA	NA	Low skin-fold thickness
GMFCS≥3	Stevenson 2006	364	9.3	NA	15.5% / NA	NA	Prior fracture High body fat Feeding tube
Quadriplegia Diplegia Hemiplegia	Leet 2006	418	10.6	NA	12% / NA	NA	Feeding tube Anticonvulsants Valproic acid Age
Duchenne							
	Larson 2000	41	10.3	-3.9	44% / 0%	NA	NA
	McDonald 2002	378	12	NA	20.9% / NA	NA	NA
	Bothwell 2003	33	11.5	NA	27% / 30%	NA	Steroid treatment
	King 2007	75	16.9	Spine -2.0	39% / 32%	NA	Steroid treatment
		68	14.4		27% / 0%		
MMC							
	Quan 1998	35	11.1	No fractures: -1.0 With fractures -3.1	23% / NA	NA	High U-Ca excretion

2.9. Bone health in adults with intellectual disability

Any long-term illness or disability in childhood may result in subnormal PBM with osteoporosis and susceptibility to fractures later in life (Sochett and Mäkitie 2005). Adults with ID living in residential care are known to be at risk for low BMD (Aspray et al. 1998, Center et al. 1998, Jaffe et al. 2005), and fractures occur 1.7-3.5 times more frequently in them than in the normal population (Tannenbaum et al. 1989, Lohiya et al. 1999). ID is often associated with other disorders, e.g. CP and other causes of reduced mobility and muscle weakness, epilepsy, feeding difficulties, and hypogonadism (Arvio and Sillanpää 2003, Bertoli et al. 2006a). In addition, adults with ID are at risk of vitamin D insufficiency due to lack of exposure to the sun, especially if institutionalized, and due to feeding difficulties and consequent nutritional deficiencies. Low BMD, as measured by QUS and defined as a BMD value more than 2 SD below young adult reference values, was found in 51% and 82% of institutionalized men (Jaffe and Timell 2003) and women (Jaffe et al. 2001), respectively, with ID.

Management of menstruation is often challenging in women with ID (Dizon et al. 2005). In Finland and in some other countries therapeutic amenorrhea induced by progestin has been a clinical practice for over 40 years (Lydecken 1966). In a recent Finnish study, this progestin- induced complete suppression of ovarian estrogen secretion was a significant risk factor for osteoporosis, suggesting that new strategies in the management of menstruation and gynecological follow-up should be considered in this population (Arvio et al. 2009).

2.10. Vitamin D

Several nutritional factors influence bone and mineral metabolism. Vitamin D has a key role in this metabolism. Optimal vitamin D status has been much debated in recent years, not only because of its role in bone health, but also due to the emergence of evidence of its function in many other tissues and association with various chronic conditions.

Vitamin D metabolism

Vitamin D is a fat-soluble vitamin. There are two forms of vitamin D: D2 or ergocalciferol, synthesized by plants, and D3 or cholecalciferol, synthesized by mammals (Holick 2004). Since the early 1920s, D2 has been produced industrially through ultraviolet exposure of foods and used as vitamin D supplementation. D3 was initially obtained from cod liver oil. Earlier, these two preparations were regarded as equal, but more recently this view has been questioned (Houghton and Vieth 2006). Today, most of the preparations used in both Finland and Europe contain D3; however, vitamin D drops used in Finland contain mainly D2. D3 is also used in fortification of foods.

In humans, vitamin D3 is synthesized in the skin with the help of ultraviolet B (UVB) radiation (Figure 5). In the skin, 7-dehydrocholesterol is transformed to previtamin D3, and further to vitamin D3, which binds to vitamin-D binding protein and is transported to the liver (Holick 2004). Vitamin D is metabolized in the liver by 25-hydroxylase enzyme to 25-hydroxyvitamin D3 (25-OHD, calcidiol). The production of 25-OHD is not significantly regulated, and activity of the circulating 25-OHD molecule depends on the amount of vitamin D binding protein. The lack of physiological regulation of 25-OHD makes its serum concentration a good indicator of vitamin D status. The half-life of 25-OHD is 20 - 30 days. (Vieth 1999, Holick 2004).

Calcidiol is hydroxylated in the kidney and other tissues to its biologically active form, 1,25 dihydroxyvitamin D3 [1,25$(OH)_2$D, calcitriol], by 1α-hydoxylase (Holick 2004). The half-life of calcitriol is only a few hours. Negative feedback of 1,25$(OH)_2$D, PTH, serum phosphate, growth hormone, and estrogen regulate the formation of calcitriol. Hypocalcemia and vitamin D deficiency increase PTH secretion; this in turn increases the production of 1,25$(OH)_2$D (Holick 2004). The active metabolite enters the target cells and binds to vitamin D receptor (VDR) in the nucleus. The binding activates genes that are regulated by vitamin D and subsequently affects their protein production (Horst et al. 2005). 1,25$(OH)_2$D regulates expression of more than 200 target genes (Cannell and Hollis 2008). Vitamin D receptors are found in almost every tissue of the body, and the effects of the vitamin are dependent upon cell type (Holick 2004).

Absorption of calcium from the small intestine is facilitated by vitamin D. In good vitamin D status, active calcium transportation increases, while in poor vitamin D status calcium diffuses only passively. In the presence of low vitamin D concentration, calcium absorption from the intestine is inadequate, resulting in reduction of serum calcium concentration (Heaney et al. 1997). Low serum calcium stimulates excretion of PTH. PTH increases the production of calcitriol by activating 1α-hydroxylase in the kidney. If 25-OHD is unavailable, calcium absorption from the intestine cannot increase. In this situation, serum calcium concentration can be maintained by mobilizing calcium from the skeleton (Heaney et al. 1997, Horst et al. 2005). This negative calcium balance has a deleterious impact on bone health and may result in rickets or osteomalacia and skeletal fragility. 25-OHD is the nutritional indicator of vitamin D homeostasis in serum because it is better correlated with calcium absorption than $1,25(OH)_2D$ in adults (Heaney et al. 1997, Horst et al. 2005).

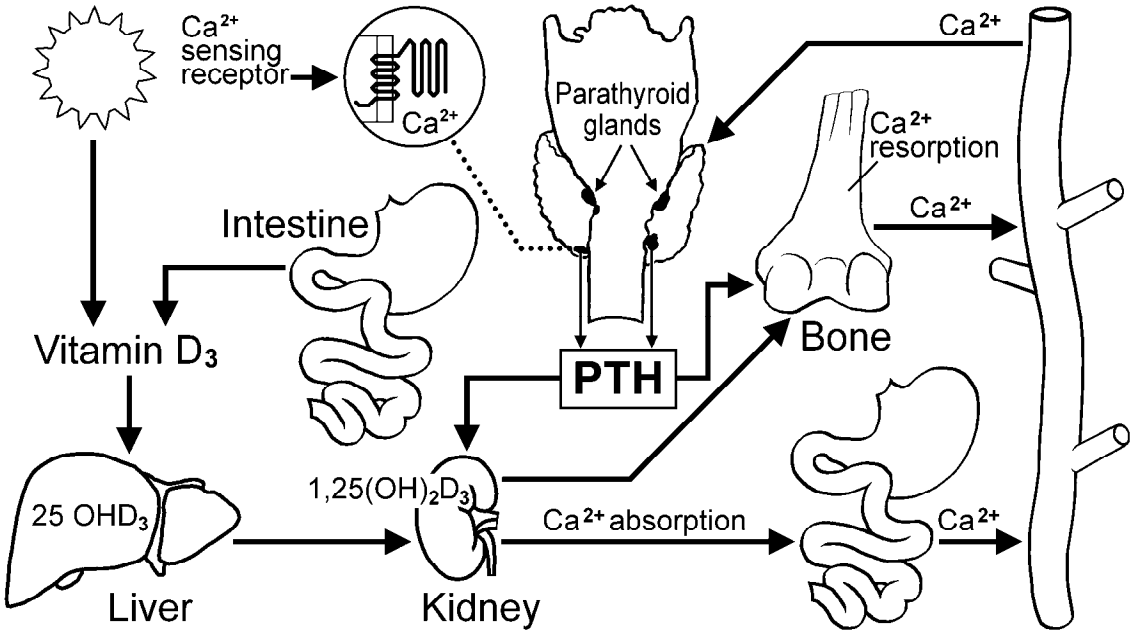

Figure 5. Vitamin D metabolism. PTH, parathyroid hormone; 25-OHD, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D.

Functions of vitamin D in the body

Skeletal effects

Vitamin D promotes calcium absorption from the intestine. Adequate vitamin D concentrations prevent bone loss and decrease fracture risk in the elderly (Dawson-Hughes et al. 1995, 1997). The optimal level for bone health in adults is >80 nmol/L (Dawson-Hughes et al. 2005). Higher 25-OHD concentration is associated with better lower-extremity function and decreased falls among elderly individuals (Bischoff-Ferrari et al. 2004a, 2004b, 2009) and better musculoskeletal function also in children (El-Hajj Fuleihan et al. 2006). A weak association between forearm BMD and S-25-OHD concentrations was found in Finnish 14- to 16-year-old females (Outila et al. 2001).

Nonskeletal effects

Conversion of 25-OHD to its active form occurs mainly in the proximal renal tubule, but also in bone, vascular smooth muscle cells, macrophages, parathyroid glands, prostate, colon, pancreas, brain, and testis. As a consequence, poor vitamin D status is associated with various chronic diseases, including type I diabetes mellitus, breast, prostate, and colon cancers, multiple sclerosis, hypertension, and heart disease, as well as with disorders of the immune system and infections (Cantorna et al. 2004, Holick 2004, Cannell et al. 2006).

Sources of vitamin D

The human body has two sources of vitamin D. It is obtained either from the diet or by cutaneous synthesis through the process initiated by ultraviolet radiation (290-315 nm) on the skin. However, UVB radiation is insufficient for adequate cutaneous vitamin D synthesis during the winter months beyond latitudes of 35° (Webb et al. 1988) (Figure 6). In Boston (42°N, corresponding to Rome in Europe) no cutaneous vitamin D synthesis was reported to occur between November and February, and in Edmonton (52°N, corresponding to London in Europe) the inactive period was even longer: from

September to March. Further south in Los Angeles, 34°N, and in Puerto Rico, 18°N, enough sunlight was adequate to photoconvert 7-hydrocholesterol to previtamin D3 even in January (Webb et al. 1988). In Finland (latitudes 60-70 °N), vitamin D synthesis can be poor even in summer months, especially in a rainy summer, in people with dark skin, those avoiding the sun or using sunscreens, and those with limited outdoor activities.

Below 35°, the angle of the sunlight is direct, and vitamin D3 synthesis can occur year-round, but above latitudes of 35° the angle is so oblique during the winter months that most UVB photons below 315 nm are absorbed by the ozone layer (Holick 2004). Further, because of the oblique angle of the sunlight, UVB radiation reaching the earth is reduced also early in the morning and in the late afternoon at higher latitudes, and very little if any vitamin D3 is produced in the skin even in the summer. In order to have vitamin D3 production in the skin, one should have sun exposure between 10 am and 3 pm during the spring, summer, and autumn (Holick 2004).

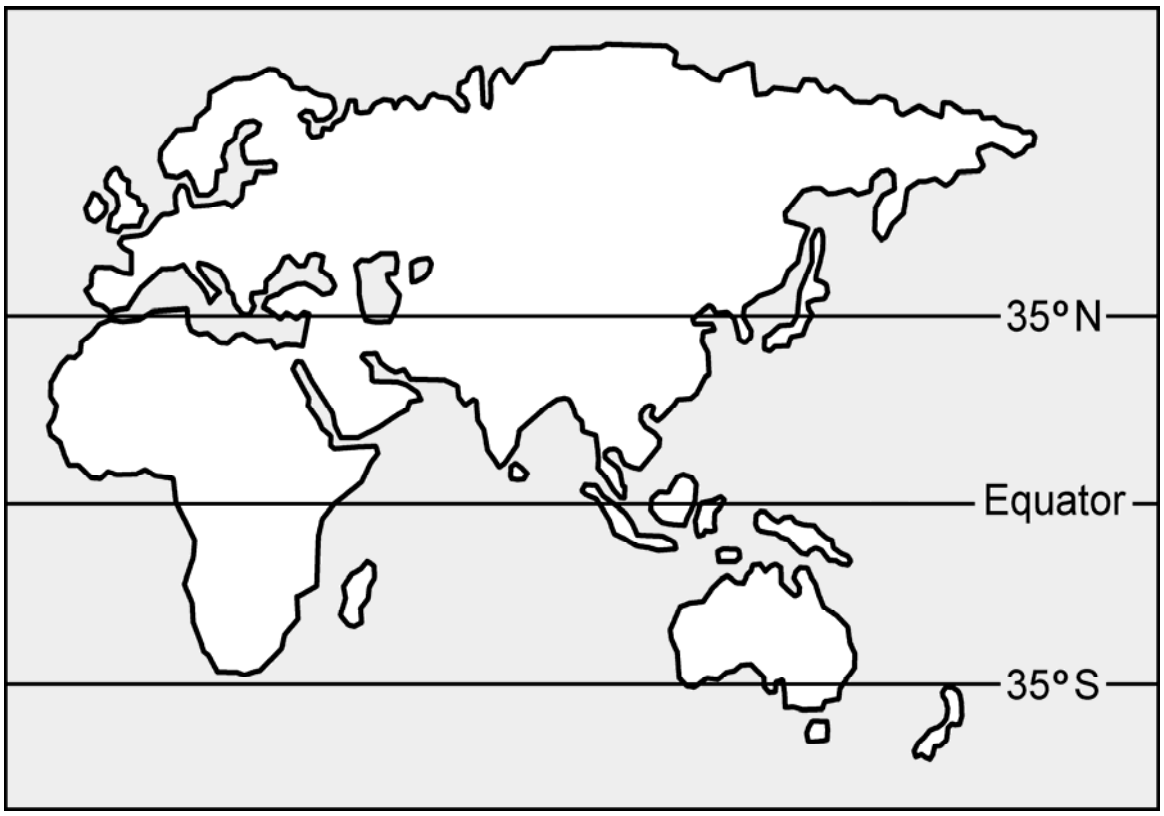

Figure 6. Map of latitudes. During the winter months vitamin D synthesis in the skin is practically absent in regions above latitudes of 35°.

The amount of 7-dehydrocholesterol and thus the capacity to produce vitamin D3 in the skin declines in the elderly; a 70-year-old makes 75% less vitamin D3 than a 20-year-old when exposed to the same amount of sunlight (Holick 2004). Sunscreens absorb UVB radiation. Sunscreen with a sun protection factor of 8 reduces the capacity of the skin to produce vitamin D3 by >95%, and with a sun protection factor of 15 the capacity is reduced by >98% (Holick 2004).

In the human body, vitamin D3 is thought to have higher bioavailability than vitamin D2. In addition, vitamin D3 is suggested to be more effective than vitamin D2 in maintaining 25-OHD values in humans (Trang et al. 1998, Armas et al. 2004). Vitamin D2 metabolites have a weaker binding affinity to vitamin D binding protein, and this leads to a shorter circulating half-life and faster clearance from circulation (Hollis 1984, Houghton and Vieth 2006). And, because not bound to binding proteins, vitamin D2 is thought to be more toxic than vitamin D3. Vitamin D3 is more stable than D2 (Trang et al. 1998). However, in a recent study by Holick et al. (2008) vitamin D2 was as effective as D3 in maintaining serum 25-OHD values.

Dietary sources of vitamin D

There are few sources of vitamin D3 in the diet. The main sources are fish, liver, egg yolk, some mushrooms, fortified margarines and spreads, and milk. Table 4 presents some food items and their vitamin D content.

The dietary guidelines for daily vitamin D intake have significantly changed over the years in Finland, from 2400-4000 IU (60-100 μg) in 1940 to 200 IU (5 μg) (in adults) in 2004. In 1964, the daily recommended dose for infants was reduced from 4000-5000 IU (100-125 μg) to 2000 IU (50 μg) (Hallman et al. 1964). The recommended dose was further reduced to 1000 IU (25 μg) in 1975, and to 400 IU (10 μg) in 1992 (Ala-Houhala et al.1995). After 2004, the recommended intake for children under 3 years and persons over 60 years, pregnant and lactating women, and other persons at risk has been 400 IU (10 μg). For persons aged 3-60, years the recommended daily intake is 300 IU (7.5 μg) (Finnish Nutrition Recommendations 2005). These guidelines are based on the Nordic Nutrition Recommendations (Nordic Council of Ministers 2004). In 2002, the

Scientific Committee on Food of the European Commission set the upper limit for daily vitamin D intake to 1000 IU (25 μg) per day for children under 11 years and 2000 IU (50 μg) per day from those aged 11 years to adulthood (Scientific Committee on Food 2002).

Because of poor dietary vitamin D intake and poor vitamin D status in Finland, the Ministry of Trade and Industry approved an increase in vitamin D fortification of foods at the beginning of 2003. Liquid dairy products, including milk, sour milk, and yoghurt, are fortified with 0.5 μg (20 IU) /100 mL vitamin D3, and margarines and spreads are fortified with 10 μg (400 IU) / 100 g. Despite these actions, a follow-up study in 2004 by Lamberg-Allardt et al. (2006) showed that vitamin D3 intake from food was insufficient in 4- to 6-year-olds, 13- to 14-year-olds, 14- to 17-year-old females, 29- to 37-year-olds, and 72- to 77-year-old females.

Table 4. Vitamin D content in selected food items. Source: National Food Database Fineli®, National Public Health Institute, Finland.

Product	**Vitamin D content (μg / 100 mg)**
Eel [1]	25.6
River lamprey [2]	25.6
Pike perch [3]	24.6
Whitefish, pollan, lavaret [4]	22.0
Baltic herring	18.0
Chantarelle	12.8
Margarine	9.2
Salmon	8.9
Tuna	7.2
Egg Yolk	6.5
Lorchel [5]	5.7
Mushroom milk caps [6]	5.3
Boletus edible [7]	2.9
Egg, boiled	2.2
Coalfish [8]	1.5
Liver	0.8
Milk	0.5
Meat	0.2-0.5
Champignon	0.2

Translation to Finnish: [1]ankerias, [2]nahkiainen, [3]kuha, [4]siika, [5]korvasieni, [6]rousku, [7]herkkutatti, [8]seiti.

Optimal vitamin D status in adults

The definition of adequate vitamin D level has been widely discussed in recent years. In adults, the optimal level of 25-OHD is now considered to be >75 or 80 nmol/L (Dawson-Hughes 2005). This level is based on the inverse relationship between S-25-OHD and S-PTH. Studies in adults suggest that PTH levels begin to increase at S-25-OHD concentrations <50 nmol/L and that S-25-OHD concentration of about 80 nmol/L is needed to plateau PTH and prevent negative effects of hyperparathyroidism on bone (Chapuy et al. 1997, Dawson-Hughes et al. 2005).

Severe vitamin D deficiency resulting in rickets in children and osteomalacia in adults is associated with S-25-OHD levels <12.5 nmol/L (Pettifor 2005). Low vitamin D levels between 12.5 and 30 nmol/L have been associated with increased fracture risk in the elderly (LeBoff et al. 1999). There is no evidence of adverse effects with S-25-OHD concentrations <140 nmol/L, and the highest S-25-OHD obtained from sunshine was 225 nmol/L in a farmer in Puerto Rico (Vieth 1999). Outdoor workers have been demonstrated to have median concentrations of 122 nmol/L at the end of summer (Barger-Lux and Heaney 2002). Hypercalcemia and 25-OHD concentrations >220 nmol/L have been regarded as signs of vitamin D intoxication in people with erroneous consumption of extremely large amounts of vitamin D or with pharmacologic use, usually vitamin D2, from 20 000 to 600 000 IU/day (Vieth 1999).

Optimal vitamin D status in children

In children, the optimal serum concentration of 25-OHD has also recently been questioned. The Lawson Wilkins Pediatric Endocrine Society has recommended that vitamin D levels >50 nmol/L in children should be regarded sufficient (Misra et al. 2008) (Table 5). According to the same recommendation, 25-OHD levels <38 nmol/L are regarded as deficient. However, some studies in children and adolescents demonstrate a similar relationship between S-25OHD and P-PTH as in adults, suggesting that the desirable level also in children should be approximately 80 nmol/L (Guillemant et al. 1999, El-Hajj Fuleihan et al. 2001).

Table 5. Vitamin D status in children and adults (modified according to Dawson-Hughes et al. 2005, and Misra et al. 2008).

Children		**Adults**	
S-25-OHD (nmol/L)	**Vitamin D status**	**S-25-OHD (nmol/L)**	**Vitamin D status**
<12.5	Severe deficiency		
<37.5	Deficiency		
37.5-50	Insufficiency	< 50	Deficiency
50-250	Sufficiency	50-79.9	Insufficiency
		>80	Sufficiency

S-25OHD, serum 25-hydroxyvitamin D.

Severe vitamin D deficiency

During childhood severe vitamin D deficiency causes rickets, muscle weakness, and hypocalcemia. Clinical symptoms are bowed legs, swollen growth plates at the end of long bones, enlargement of costochondral junctions in ribs, poor growth, delayed motor development, and hypotonia (Pettifor 2005). Rickets can only occur in the presence of unfused epiphyses. Therefore adults cannot suffer from rickets. In adults, vitamin D deficiency causes osteomalacia, which is characterized by failure of normal mineralization (Allgrove 2009).

2.11. Calcium and bone health

Calcium is essential for normal bone health at all ages. During childhood calcium is needed for skeletal growth and in adulthood for maintenance of bone mass and content. Inadequate calcium intake results in decreased calcium absorption in the intestine, low levels of ionized calcium, and increased secretion of PTH (Broadus 2003). PTH regulates blood calcium levels by releasing calcium from bone and also by stimulating

calcium reabsorption in the kidney. PTH also stimulates the renal synthesis of $1,25(OH)_2D$, thus increasing intestinal calcium absorption (Broadus 2003).

Dietary calcium has been shown to have beneficial effects on bone mass at all ages, although the results are not always consistent. Findings in studies concerning dietary supplementation of calcium and its effects on BMD and fracture risk later in life are controversial. Results are dependent on various factors, including the amount of calcium supplement used, the timing of supplementation, and pubertal stage, and whether the study protocol used vitamin D supplement or not. Higher calcium intakes have been related to higher bone mass in children, young adults, and postmenopausal women in observational studies (Heaney 2000). Clinical trials with calcium supplements have also shown positive effects on bone mass in children and adolescents (Matkovic et al. 2004). Further, a positive correlation between consumption of dairy products in childhood and also in adulthood and higher BMD in adulthood has been observed (Murphy et al. 1994, Soroko et al. 1994, Kalkwarf et al. 2003). It is more beneficial for bone mass accrual to increase calcium intake by dairy products than by tablets containing a similar amount of calcium. Calcium is thought to be better absorbed in lower amounts from dairy products throughout the day than from larger amounts in pills twice a day (Cheng et al. 2005). Some studies have suggested that calcium supplements have no or only a small effect on BMD in childhood or on fracture risk later in life (Winzenberg et al. 2006).

The dietary recommendations for daily calcium intake in Finland are 540 mg in children under one year, 600 mg in children from one to five years, 700 mg in children from six to nine years, 900 mg in adolescents from 10 to 17 years, and 800 mg in adults (Finnish Nutrition Recommendations 2005).

Table 6. Calcium content in some food items. Source: National Food Database Fineli®, National Public Health Institute, Finland.

Product	Calcium content (mg / 100 g)
Cheese	800
Nettle[1]	590
Sardine	430
Baltic herring	340
Soya beans	160
Milk	180
Yoghurt	150
Ice cream	150
Rainbow trout[2]	130

Translation to Finnish: [1]nokkonen, [2]kirjolohi.

2.12. Other nutritional factors and bone health

Adequate nutrition plays a major role in the prevention and treatment of osteoporosis. The most important micronutrients for bone health are vitamin D and calcium. However, several other minerals and vitamins, such as proteins, magnesium, phosphorus, potassium, fluoride, iron, copper, manganese, zinc, and vitamins A, K, and C are required for bone formation (Nieves 2005, Palacios 2006).

Protein is a part of the organic matrix of bone collagen structure. Proteins are also essential for production of hormones and growth factors involved in bone synthesis. Better protein intake has been shown to increase insulin-like growth factor I (IGF-I), which is osteotrophic (Palacios 2006). Inadequate protein intake is known to negatively affect bone health in the elderly by diminishing the ability to repair fractures. The suggested daily protein intake is 0.8 g/kg. Better nutritional status has been found to associate with better BMD also in children with motor disability (Henderson et al. 2005).

3. AIMS OF THE STUDY

The aim of this study was to evaluate factors associated with bone health in children with severe motor disability and in adults with intellectual disability. Specific objectives were:

1. to assess bone mineral density and prevalence of fractures,
2. to evaluate nutritional and other factors contributing to impaired bone health,
3. to analyze vitamin D status, and
4. to determine optimal vitamin D supplementation

in children with severe motor disability and in adults with intellectual disability.

4. PATIENTS AND METHODS

4.1. Study subjects

Children with severe motor disability at Päijät-Häme Central Hospital

The study group (study **I, II**) comprised Finnish children with severe motor disability aged 5 - 16 years, who were followed at Päijät-Häme Central Hospital, Lahti, Finland. The hospital serves as a referral center for a population of 210 000 (34 000 aged <16 years) in southern Finland. Approximately 1350 children are followed at the Department of Pediatric Neurology annually. Of the 64 eligible patients with severe motor disability, 59 (92%) consented to participate. The study included children with severe CP (N=36), MMC (N=7), neuromuscular disorders [including Duchenne muscular dystrophy (DMD), and SMA] (N=5), or various syndromes causing motor disability (N=11), and at least level II disability on the GMFCS. The study was carried out between 2005 and 2007.

Children with severe motor disability at Ruskeasuo School

Study **III** involved children and adolescents at Ruskeasuo School, the Developmental Center for Disabled Children, which is a state-owned school for children with motor disabilities, in Helsinki, Finland. Forty-four of the school's 114 students in spring term 2005 consented to participate in the prospective study. Neither vitamin D nor nutritional status, or the severity or the nature of the underlying condition was used as a selection criterion. The final study group included 25 males and 19 females, ranging in age from 9 to 18 years.

Adults with intellectual disability at Pääjärvi Nursing Homes

Finland was divided into 16 regional special welfare districts offering public services to inhabitants with ID in 2005. Pääjärvi Inter-Municipal Association was one of the districts. Pääjärvi, located in Lammi, southern Finland, served as a referral center for a population of 340 000. All adult residents (N=164) of the Pääjärvi Nursing Homes were

invited to participate (study **IV**). Altogether 145 residents (88%) consented to participate, 138 of whom completed the study. The study commenced in January 2005.

4.2. Methods

Study design

The study protocols were approved by the Hospital Research Ethics Committees of Päijät-Häme Central Hospital (**I, II, IV**) and Helsinki University Hospital (**III**). A written informed consent was obtained from all children with motor disability and from adults with ID, or from their parents/legal guardians. The National Agency for Medicines was informed of the protocol of Study **IV**. Table 7, page 51, summarizes the methods used in Studies **I-IV**.

In Studies **I and II**, the subjects were clinically examined at their annual follow-up visit at the Department of Pediatric Neurology, Päijät-Häme Central Hospital. Blood samples were collected and imaging studies carried out during the hospital visit. Clinical data were collected from hospital records and other information related to health and nutrition by questionnaires.

Two intervention studies with vitamin D were carried out at Ruskeasuo School (**III**) and at Pääjärvi (**IV**). Study **III** started by obtaining blood samples in the first week of March. The study cohort was then divided into two groups (Treatment group, N=21, and Control group, N=23) based on the measured baseline S-25-OHD concentration: the S-25-OHD values of each study subject were arranged in increasing order, and every other child was assigned to receive vitamin D. After this baseline evaluation, the Treatment group was given 1000 IU (25 µg) of vitamin D3 (2.5 mL of Deetipat 400 IU/mL ®, Ferrosan, Espoo, Finland) perorally daily five days per week for 10 weeks. Peroral vitamin D was given by school staff during the school lunch to ensure compliance. The Control group (N=23) continued with their normal lunch program at school. All participants were advised to continue their regular diets and possibly ongoing additional vitamin supplements. Blood samples were obtained from all subjects at the end of the 10-week intervention period in late May.

In Study **IV**, the subjects were allocated to receive vitamin D3 either perorally 800 IU (20 µg) per day for six months (cumulative dose approximately 144 000 IU) (PO group) or as a single intramuscular injection of 150 000 IU (3750 µg) (D3-Vicotrat® 100 000 I.E. /1mL, Heyl Corp., Berlin, Germany) (IM group). Allocation was based on vitamin D status and gender only, aiming at similar baseline vitamin D and gender distribution in the two intervention groups. Peroral calcium (1000 mg/day) was given to all study subjects. The blood samples were repeated six months later. Only 138 (85%) subjects completed the study (72 in the PO group and 66 in the IM group); their results were used in the analysis.

Clinical assessment

Complete medical and surgical records were obtained. Parents filled detailed questionnaires on feeding difficulties, physical activity, and time spent outdoors and kept a three-day food diary (**I, II**). Height was measured in supine position and expressed in SD units (Z-score) by comparing the values with age-, and sex-specific normative data (Sorva et al. 1990). Weight in light clothing was measured with a digital scale and expressed as a percentage of sex- and height-specific reference values (**I, II**) (Sorva et al. 1990). Body mass index (BMI) was calculated as [weight / (height) ^{2}] and expressed in kg/m^2. Pubertal maturation was assessed according to Tanner (1962) (**I, II**).

Laboratory tests

Blood biochemistry was obtained for plasma (P) /serum (S) total calcium (P-Ca), phosphate (P-Pi), P-ALP, P-PTH, S-25-OHD, creatinine (P-Crea), S-IGF-I, and insulin-like growth factor binding protein 3 (S-IGFBP3), serum transferrin, prealbumin (S-Prealb), vitamin A, vitamin E, and for complete blood count, including hemoglobin concentration (B-Hb). In Study **I**, serum estradiol or testosterone, follicle-stimulating hormone, and lutenizing hormone were obtained for girls >8 years and for boys >10 years of age. Urine (U) was obtained for analysis of creatinine (U-Crea) and calcium (U-Ca).

P-Ca, S-Ca-ion, P-Pi, P-ALP, P-Prealb, P-ALT, P-Crea, serum transferrin, S-Prealb, blood counts, U-Crea and, U-Ca were determined by standard assays. S-25-OHD was determined by radioimmunoassay in Studies **I** and **II** (IDS Inc., Fountain Hills, AZ, USA) and by high-performance liquid chromatography in Studies **III** and **IV**. The inter-assay coefficient of variation (CV) in Studies **I** and **II** at the level of 31 nmol/L was 5.1% and at 110 nmol/L, 6.0%. In Study **III**, the intra-assay CV at 21.6 nmol/L was 5.6% and at 63.8 nmol/L, 5.3%, and the inter-assay CV at 16.4 nmol/L was 7.3% and at 47.5 nmol/L, 6.3%. In Study **IV**, the intra-assay CVs were 8.9% at 30 nmol/L and 5.9% at 100 nmol/L, and inter-assay CVs 12.8% at 35 nmol/L and 8.8% at 105 nmol/L. An immunoluminometric assay was used to analyze P-PTH. The reference range for P-PTH was 0.8-7.5 pmol/L (8-75 ng/L) (**I-IV**).

S-PINP and S-ICTP were measured by competitive Radio Immuno Assay (UniQ PINP/ICTP RIA ®, Orion Diagnostica, Espoo, Finland) (**III**). The reference ranges for S-PINP and S-ICTP were age- and sex-dependent, and were for S-PINP from 400 to 800 µg/L and for S-ICTP from 4 to 20 µg/L (Crofton et al. 1997).

Radiological evaluation

Assessment of bone age and spinal radiographs

Hand-wrist x-rays were obtained to determine bone age (**I**) (Greulich and Pyle 1959). Standard anteroposterior and lateral spinal radiographs were obtained (**I**) and evaluated for vertebral morphology according to a pediatric scoring method (Mäkitie et al. 2005). This method was developed for evaluation of vertebral morphology and vertebral fractures in childhood secondary osteoporosis. The morphological changes were classified as normal anterior wedge deformities (grade 0, 1a, 1b, and 1c) when only the anterior part of the vertebra was compressed. The changes were considered abnormal compression deformities if also the middle and/or posterior part of the vertebra was compressed. These abnormal changes were further classified as mild (2a; anterior height reduction 20-49%) or severe (2b; anterior height reduction ≥ 50%) anterior wedge deformities, or mild (3a; middle height reduction 20-49%) or severe (3b, middle height reduction ≥ 50%) compression deformities (Mäkitie et al. 2005).

Bone densitometry

BMD of the lumbar spine L2-L4 was measured with DXA (Lunar Prodigy 2000, GE Lunar Corp., Madison WI, USA) (**I**). Standard scanning procedures were used. Most patients received diazepam or midazolam prior to the measurement to minimize motion artifact. Areal BMD (aBMD) values were transformed into Z-scores by comparing them with age- and sex-specific reference values (van der Sluis 2002). To correct aBMD values (g/cm^2) for bone size, apparent volumetric bone mineral density BMAD (g/cm^3) values were calculated from aBMD using the equation BMAD = aBMD x [4/ (π x width)] (Kröger et al. 1993). In this equation, width signifies the mean width of the measured area of the vertebral column (L2-L4).

The calculated BMAD values were transformed into Z-scores by comparing them with age- and sex-specific reference values (van der Sluis et al. 2002). Bone age instead of chronological age was used for all BMD values when determining Z-scores if calendar age and bone age differed by more than one year. The lumbar BMD values were omitted from the analyses if compression deformities were present in the measurement area. In MMC patients, the lumbar BMD was analyzed using the measurement area outside the lumbar defect.

Nutritional assessment

Parents or guardians (**I, II**) or school staff members (**III**) were asked to keep three-day or one-week food diaries, recording the trade names and amounts of all consumed food items and beverages. On the basis of these recordings, the average daily nutrient intakes were calculated for each patient using the computer program DIET32, version 1.4.4.1 (Aivo Finland Corp., Turku, Finland). The median energy and various nutrient intakes were calculated and compared with age- and sex-specific national nutrition guidelines (Finnish Nutrition Recommendations 2005).

Table 7. Methods used in Studies I-IV.

	Study I	Study II	Study III	Study IV
DXA	x			
Vertebral morphology	x			
Bone age X-ray	x			
Food diary	x	x	x	
Pubertal stage	x			
BMI	x	x		x
Biochemical parameters	x	x	x	x
Vitamin D intervention			x	x

Statistical analysis

Statistical analyses were performed with Statistical Package for Social Sciences for Windows version 13.0 (**III**) and version 15.0 (**I, II, IV**) (SPSS Inc., Chicago, IL, USA). A P-value of less than 0.05 was considered significant. Mann-Whitney U-test (**I - III**) and T-test (**IV)** were used to compare variables between the groups. Chi-square test was used for categorical data (**I, II, IV**). Pearson's correlation coefficient was used to assess correlations (**I - III**). Wilcoxon signed-ranks test was applied to assess longitudinal changes within groups (**III**). In Study **I,** possible predictors for fractures and low BMAD were evaluated by logistic regression analysis.

5. RESULTS

5.1. Bone health and its determinants in children with motor disability (I, II)

Bone mineral density

DXA results were corrected for pubertal development by using bone age instead of calendar age in 11 patients (19%) with advanced bone age and in 11 patients with delayed bone age. The median Z-score for lumbar spine BMD was -1.7 (range -3.7 - +1.0) and for lumbar spine BMAD -1.0 (range -5.0 – +2.0). The BMD Z-score was <-2.0 in 21 children (39%) and the BMAD Z-score was <-2.0 in 11 children (20%). No significant correlations were found between lumbar spine BMAD Z-score and S-25-OHD, P-PTH, P-Ca, or P-Pi levels or calcium and vitamin D intakes.

The median BMAD Z-score did not differ between those with moderate motor disability (N=26) (GMFCS II or III; -0.6, range -3.0 - +1.0) and those with severe motor disability (N=33) (GMFCS IV or V; -1.0, range -3.0 - 1.8; P = 0.28). The median BMAD Z-score in children with antiepileptic medication (N=28) was -0.7 (range -4.8 -+1.8) and in those without medication (N=31) -1.0 (range -3.0 -+1.0) (P = 0.63).

Fractures

Altogether 10 children (17%) had sustained 14 peripheral fractures (1-3 fractures each) (Table 8). Most of the fractures (in 6 subjects) occurred between 4 and 12 years and involved the lower limbs (four femur and three tibia fractures). All of these fractures occurred after a minor trauma, such as straining a leg (2), falling from a chair (4), or stumbling on the floor (4), or without a known trauma.

Compression fractures were found in 14 of the 55 patients (25%) for whom radiographs were available (Figure 7). Only one child had been diagnosed with a spinal fracture before the study. Most of the compression fractures were in the thoracic region. One

boy with DMD had multiple thoracic fractures and one lumbar fracture. In eight subjects, the fractures were classified as mild anterior wedge deformities (grade 2a), in two subjects as severe anterior wedge deformities (grade 2b), and in four subjects as mild compression deformities (grade 3a). Peripheral fractures were also seen in four patients with grade 2a (2 patients), 2b (1 patient), or 3a (1 patient) thoracic fractures.

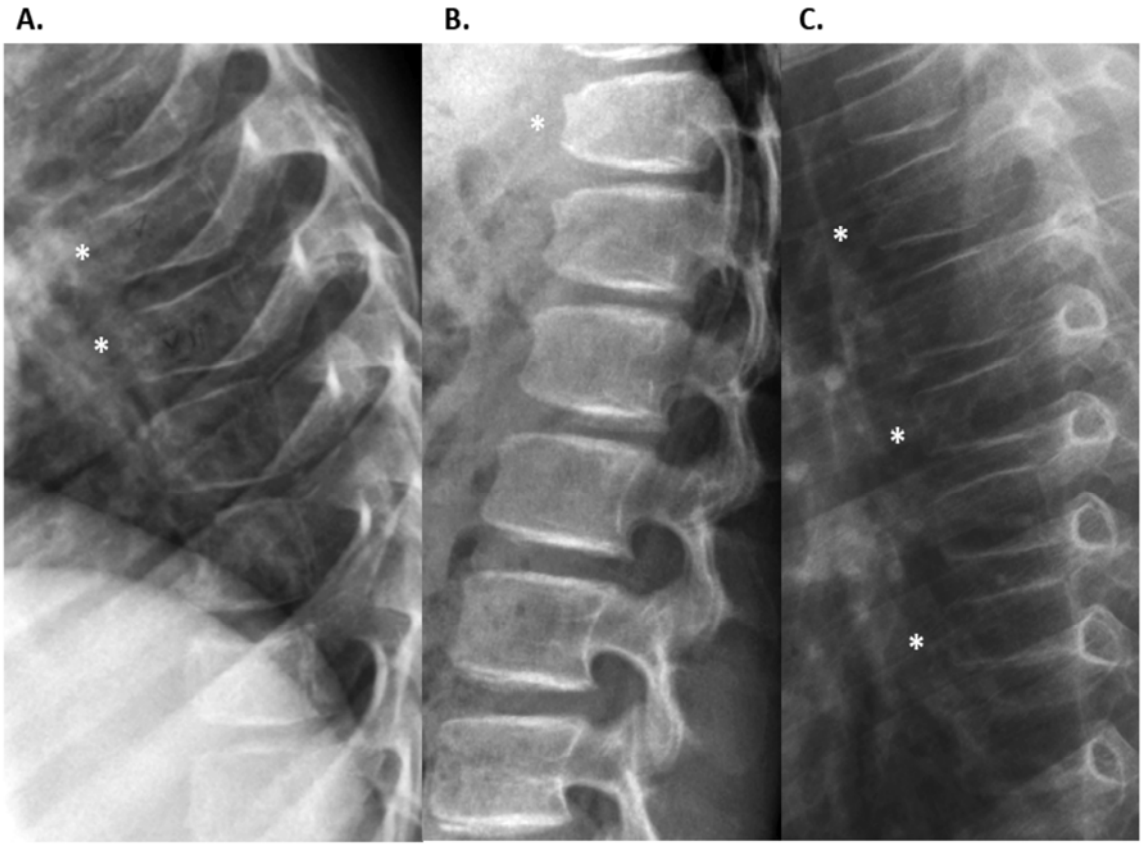

Figure 7. Compression fractures in three children with motor disability. (A) Two grade 2a compression fractures. (B) One grade 2a compression fracture. (C) Three compression fractures (2a, 3a, and 3a). Compressed vertebrae are marked with asterisks. Reprinted from Kilpinen-Loisa et al. 2009 with the permission of Wiley-Blackwell**.**

Table 8. Etiology of disability and presence of fractures.

Diagnosis	Patients with fractures		Spinal fractures	Peripheral fractures
	N	%		
Cerebral palsy	9/36	25	7	5
Neuromuscular disorder	5/5	100	4	1
Myelomeningocele	1/7	14	NA	1
Chromosomal anomaly	4/9	45	2	3
Unknown	1/2	50	1	-

NA, not assessed

No differences were observed in age and gender distribution or in anthropometric characteristics in children with (N=20) and without (N=39) spinal and/or peripheral fractures. The median BMAD Z-score was significantly lower in patients with fractures (-1.7, range -5.0 – 0.0) than in those without fractures (-0.6, range -2.9 - +1.8) (P = 0.004).

In logistic regression analyses, both low BMAD and hypercalciuria were significant predictors for fractures, with OR 9.82 (95% CI from 1.32 to 72.89) and OR 8.21 (95% CI from 1.32 to 51.05), respectively.

Diagnosis of osteoporosis

According to the previously established criteria for osteoporosis in children (Rauch 2008), the diagnosis could be made for 10 of the 59 children (17%). Table 9 shows the clinical characteristics of these 10 children.

Table 9. Characteristics of 10 subjects with a diagnosis of osteoporosis. * used a vitamin D supplement; ** % of age-specific recommendation; BMAD, bone mineral apparent density; BMI, body mass index; DMD, Duchenne muscular dystrophy; GMFCS, gross motor function classification system; MMC, myelomeningocele; NA, not available; SMA, spinal muscular atrophy; S-25OHD, serum 25-hydroxyvitamin D.

Age (yrs)	**Gender**	**Etiology**	**GMFCS**	**BMI ($kg/m^{2)}$**	**Epilepsy**	**Vitamin D intake (µg)**	**S-25OHD (nmol/L)**	**Hyper-calciuria**	**Energy intake (%) ****	**BMAD Z-score**	**Peripheral / Vertebral fractures**
9.8	F	MMC	III	15.5	no	NA	32	no	NA	-3.5	yes / no
6.3	M	Tetraplegy	V	15.6	yes	NA	104	no	NA	-3.4	no / yes
12.9	M	DMD	IV	14.2	no	5.5	34	yes	74	-3.4	no / yes
5.7	M	Hemiplegy	IV	15.9	yes	4.5	57	no	82	-3.3	yes / yes
13.5	F	Chromosomal anomaly	V	18.4	yes	9.0	85	yes	42	-3.1	yes / yes
11.3	M	Tetraplegy	V	13.6	no	7.1 *	40	no	55	-2.9	yes / yes
5.5	M	Chromosomal anomaly	IV	17.8	yes	14	83	yes	75	-2.8	no / yes
11.1	F	SMA	IV	19.9	no	8.0 *	72	yes	58	-2.6	yes / NA
13.2	M	Diplegy	II	15.2	no	NA	57	no	NA	-2.0	yes / no
13.2	M	Chromosomal anomaly	IV	13.5	yes	6.7	71	no	54	-2.0	no / yes

Biochemical findings

The biochemical values in 54 children are presented in Table 10. The median S-25-OHD was 45 nmol/L. S-25-OHD was consistent with vitamin D insufficiency (<50 nmol/L) in 20 patients (34%) and with vitamin D deficiency (<37.5 nmol/L) in 15 (25%) patients. Only 4 patients (7%) had a S-25-OHD value >80 nmol/L. P-PTH was supranormal in 5 children (8%). The medians for the other biochemical parameters were within the normal range. All hormonal values were appropriate for Tanner stage.

Table 10. Biochemical findings in 54 children with severe motor disability. Results as medians (range).

Measured parameter	Results	Reference range
S-25-OHD (nmol/L)	45.0 (18-85)	>80
P-PTH (pmol/L)	4.0 (1.2-33.0)	1.0-7.5
P-Ca (mmol/L)	2.38 (2.04-2.75)	2.05-2.70
P-Pi (mmol/L)	1.44 (0.91-1.70)	1.10-1.80
P-ALP (U/L)	210 (40-719)	115-445
U-Ca/U-Crea (mmol/L /mmol/L)	0.33 (0.02-2.91)	<0.70
S-IGFBP3 (mg/L)	3.7 (1.4-6.0)	1.0-9.6
S-IGF-I (nmol/L)	18 (4-57)	7-129

P-ALP, plasma alkaline phosphatase; P-Ca, plasma calcium; U-Ca, urine calcium; U-Crea, U-creatinine; S-IGF-I, serum insulin-like growth factor I; S-IGFBP3, serum insulin-like growth factor binding protein 3, P-Pi, plasma phosphate; P-PTH, plasma parathyroid hormone; S-25OHD, serum 25-hydroxyvitamin D.

Hypercalciuria

Altogether 16 children had hypercalciuria (U-Ca/U-Crea ≥ 0.70): 10 (50%) of those with any fracture and 6 (15%) of those without fractures (P = 0.01). There were no significant differences in the median intakes of calcium or the median BMD Z-scores between the groups with or without hypercalciuria. However, the median BMAD Z-score was significantly lower in children with hypercalciuria than in children without

hypercalciuria (-1.2 vs. -0.7, P = 0.03). Presence of hypercalciuria was identified as an independent risk factor for fractures (OR 7.16; 95% CI from 1.65 to 31.05).

Nutrition

Three-day food diaries were obtained from 54 subjects (Study **II**). Results of nutrient intakes essential for bone health are presented in Table 11. The median calcium intake, including supplements, was 142% of the recommended dietary intake, or 1123 mg (range 417-2051 mg). Four children received calcium supplementation of 400-500 mg/day. The daily calcium intake was on average good, and the median intake exceeded the recommendation in all age groups, but only in the youngest age group did all children receive sufficient calcium. Vitamin D intake was poor, 5.9 μg/day (range 2.5-20 μg/day), which is only 76% of the recommended intake. This vitamin D intake included vitamin D supplementation 2.5-10 μg/day in nine children. Intake of phosphate and vitamin A tended to be high.

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Table 11. Proportions of energy and nutrient intakes in children with motor disability. The recommendation and the results (median and range) are given separately for each age group. E%, share of total energy as a percentage; FNR, Finnish nutrition recommendations.

	Energy (kcal)	**Energy (E%)**	**Protein (g)**	**Protein (E%)**	**Vitamin D (µg)**	**Calcium (mg)**	**Phosphorus (mg)**
Age 2-5 y, FNR	1625			10-15	7.5	600	470
N=9	1339 (1150-1949)	82 (70-119)	55 (30-81)	16 (10-21)	4.5 (2.7-14)	863 (679-1391)	1084 (593-1560)
Age 6-9 y, FNR	1790				7.5	700	540
N=13	1657 (975-1887)	92 (53-105)	71 (34-90)	17 (14-20)	5 (2.5-14)	1163 (417-1700)	1261 (741-1775)
Girls 10-15 y, FNR	2010-2150				7.5	900	700
N=15	1687 (900-2020)	79 (42-100)	66 (25-103)	16 (11-21)	9 (3-18)	1320 (540-2051)	1428 (450-2188)
Boys 10-15 y, FNR	2340-2700				7.5	900	700
N=17	1522 (963-2328)	65 (41-99)	61 (38-95)	17 (12-20)	6 (3-20)	1123 (464-1697)	1343 (859-2094)
All age groups total intake (%) FNR		100			100	100	100
N=54		**76**		**17**	**76**	**142**	**202**

The proportions of energy intake were similar in the group of children with an energy intake of over 80% and in those with an energy intake less than 80% of the age-specific recommendation (Table 12). The shares of protein and fat intake exceeded recommendations (recommendation for protein intake is 10-15E% and for fat intake <30E%) in both groups. BMD Z-score was significantly lower in children with a smaller energy intake, while no difference was present in BMAD Z-scores. Fractures were more common in children with lower energy intake. Intakes of calcium were also smaller in children with low energy intake.

Table 12. Differences in characteristics between children with energy intake <80% and ≥80% of age-specific recommendation. Results presented as medians (range).

	Energy intake < 80% of recommendation N=31	**Energy intake ≥ 80% of recommendation N=23**	**P-value**
Fractures (N)	12 (39%)	4 (17%)	0.08
BMAD Z-score	-1.1 (-4.8-1.8)	-0.5 (-3.3-1.4)	0.19
BMD Z-score	-2.0 (-3.0-1.0)	-1.3 (-4.0-1.0)	0.04
GMFCS score	4.0 (2-5)	3.0 (2-5)	0.01
Height Z-score	-1.5 (-6.5-0.2)	-0.8 (-3.0-0.7)	0.02
Adjusted weight (%)	-8 (-33-105)	-3 (-22-32)	0.69
BMI (kg/m^2)	14.5 (11.4-36.7)	15.7 (14-23)	0.55
S-25-OHD (nmol/L)	41 (18-85)	49 (32-73)	0.41
P-PTH (pmol/L)	4.3 (1.2-33)	3.4 (1.8-9.9)	0.35
S-Ca (mmol/L)	2.42 (2.07-2.75)	2.37 (2.00-2.50)	0.16
B-Hb (g/L)	134 (104-152)	129 (113-156)	<0.01
S-Prealb (g/L)	0.21 (0.10-0.30)	0.19 (0.12-0.31)	0.43
Intakes of nutrients			
Carbohydrates (E%)	51 (30-68)	49 (28-59)	0.12
Protein (E%)	16 (10-21)	17 (13-21)	0.22
Fat (E%)	31 (19-52)	33 (20-54)	0.34
Saturated fat (E%)	12 (4-19)	13 (5-21)	0.27
Vitamin D (µg)	6 (2.5-20)	7 (3-15)	0.18
Iron (mg)	8.5 (4.6-18.4)	10.0 (5.8-26.4)	<0.01
Phosphorus (mg)	1103 (450-2063)	1459 (873-2188)	<0.01
Fiber (g)	11.8 (4.8-31.6)	14.8 (8.2-21.7)	<0.01
Calcium (mg)	878 (417-1697)	1320 (714-2050)	<0.01

BMAD, bone mineral apparent density; BMD, bone mineral density; BMI, body mass index; S-Ca, serum calcium; GMFCS, gross motor function classification system; B-Hb, blood haemoglobin concentration; S-Prealb, serum prealbumin; P-PTH, plasma parathyroid hormone; S-25OHD, serum 25-hydroxyvitamin D

5.2. Vitamin D interventions (III, IV)

Serum 25-OHD concentrations were low in all groups at baseline: vitamin D deficiency (defined as <37.5 nmol/L in children and <50 nmol/L in adults) was present in 36% of children and 77% of adults. After the two-month intervention period, no children had vitamin D deficiency in the treatment group, while 52% of the untreated children were deficient at the beginning of the summer. In adults with ID, the six-month supplementation resulted in vitamin D deficiency in 1% and 24% of subjects in the PO and IM groups, respectively. After the intervention, S-25-OHD levels were over 80 nmol/L in 19% of treated children. The corresponding figures in adults were 42% in the PO group and 12% in the IM group. The distribution of vitamin D status in children with motor disability and adults with ID is presented in Table 13.

Table 13. Distribution of vitamin D status before and after interventions in Studies **III** and **IV**.

	Children (III)			Adults (IV)		
	Whole group (N=44)	PO group (N=21)	No supplement (N=23)	PO+IM group (N=138)	PO group (N=72)	IM group (N=66)
S-25-OHD (nmol/L)	Baseline	After	After	Baseline	After	After
< 12.5				**1%** (1)		
12.5-37.9	**36%** (16)		**52%** (12)	**46%** (64)		
38-49.9	**25%** (11)	**29%** (6)	**22%** (5)	**30%** (42)	**1%** (1)	**24%** (16)
50-79.9	**34%** (15)	**52%** (11)	**26%** (6)	**21%** (29)	**57%** (41)	**64%** (42)
>80	**5%** (2)	**19%** (4)		**2%** (2)	**42%** (30)	**12%** (8)

IM, intramuscular; PO, peroral; S-25OHD, serum 25-hydroxyvitamin D

Vitamin D intervention in children with motor disability

Biochemical parameters in the intervention studies at baseline and after interventions are presented in Table 12. At baseline, the median S-25-OHD in Study **III** was 44 (range 26 – 82) nmol/L. The median P-PTH was 33 (6–106) ng/L; it was above 60 ng/L in only one patient. P-PTH tended to be higher in patients with S-25-OHD less than 50 nmol/L than in those with greater S-25-OHD, but the correlation was not statistically significant. None of the subjects had subnormal P-Ca. P-ALP was within age- and sex-specific reference values in all study subjects. S-ICPT was above age-specific reference values in nine subjects (20%), and S-PINP in one subject; all other values were normal.

In the Treatment group, the 10-week supplementation resulted in a significant increase in the median S-25-OHD concentration, from 46 to 56 nmol/L (P = 0.012). A modest but statistically nonsignificant decrease in mean P-PTH concentration was observed. In the Control group, the median S-25-OHD level even decreased significantly during the intervention period. No significant changes were observed in P-PTH. At the end of the intervention period, S-25-OHD concentrations differed significantly in the two groups, while no other significant differences were observed in biochemical parameters. The increased S-25OHD level in the Treatment group was not associated with an increase in P-Ca concentration; P-Ca remained within the normal range in all subjects throughout the study. No differences between the groups were observed in bone turnover markers.

Vitamin D intervention in adults with intellectual disability

In Study **IV**, no significant differences in biochemical values of adults were seen between the PO and IM groups at baseline (Table 14). The means for S-25-OHD were 40 nmol/L and 41 nmol/L (P=0.62), and for P-PTH, 51 ng/L and 54 ng/L (P=0.43), in the PO and IM groups, respectively. Mild hypercalcemia without clinical symptoms was present in 0 and 2 patients and hyperphosphatemia in 3 and 4 patients in the PO and IM groups, respectively.

In the PO group, the mean S-25-OHD at 6 months was 82 nmol/L (P<0.001) for the change from baseline). P-PTH decreased significantly and was on average 33 ng/L (P<0.001); four patients had supranormal P-PTH, while P-PTH was <12 ng/L in five. S-Ca and S-Pi also increased significantly (P<0.05); S-Ca was supranormal in six (8%) and S-Pi was supranormal in 10 (14%). S-ALP decreased significantly and was on average 74 U/L at 6 months (P<0.05).

In the IM group, a significant improvement in S-25-OHD was also observed: the mean S-25-OHD increased from 41 nmol/L to 62 nmol/L (P<0.001). Eight subjects (12%) had a S-25-OHD concentration >80 nmol/L and one >100 nmol/L, while 16 subjects (12%) had S-25-OHD <50 nmol/L. The mean P-PTH decreased significantly to 34 ng/L (P<0.001); it remained supranormal in one patient and was <12 ng/L in three subjects. S-Ca and S-Pi increased significantly (P<0.05), but no supranormal S-Ca values were measured; S-Pi was supranormal in 14 patients (21%). S-ALP remained unchanged.

After the treatment, 38 patients (27%) had S-25-OHD >80 nmol/L and 15 (11%) had S-25-OHD >100 nmol/L. Three of those with S-25-OHD >100 nmol/L had P-PTH <12 ng/L, all in the PO group. S-25-OHD >100 nmol/L was associated with hypercalcemia in 2 and hyperphosphatemia in 6 subjects. Subjects with S-25-OHD >100 nmol/L had smaller BMI and were lighter than subjects with lower S-25-OHD, the mean BMI being 20.5 kg/m^2 and 24.4 kg/m^2 (P=0.017) and the mean weights 51.9 kg and 67.5 kg (P=0.002), respectively.

Table 14. Biochemical parameters at baseline and after interventions (Studies **III-IV**). Values are presented as means (SD). P-ALP, plasma alkaline phosphatase, P/S-Ca, plasma/serum calcium; IM, intramuscular group; P/S-Pi, plasma/serum phosphate; PO, peroral group; P-PTH, plasma parathyroid hormone; S-25OHD, serum 25-hydroxyvitamin D. * refers to significant difference between the groups after the intervention.

	Children (III) in March		**Children (III) in May**		**Adults (IV) in January**		**Adults (IV) in June**	
	Baseline		**After treatment**		**Baseline**		**After treatment**	
	Treatment group N=21	Control group N=23	Treatment group	Control group	PO group N=72	IM group N=66	PO group	IM group
S-25-OHD (nmol/L)	46.9 (16.2)	49.4 (27.2)	60.1 (16.1)	41.8 (14.0)*	39.9 (13.5)	41.0 (12.5)	81.6 (20.7)	61.6 (15.1)*
P-PTH (ng/L)	36.1 (20.6)	32.0 (11.8)	32.0 (19.9)	33.0 (14.1)	50.9 (23.0)	54.2 (26)	33.2 (18.0)	33.9 (15.9)
P/S-Ca (mmol/L)	2.39 (0.09)	2.41 (0.07)	2.43 (0.08)	2.43 (0.05)	2.28 (0.08)	2.30 (0.10)	2.35 (0.12)	2.33 (0.09)
P/S-Pi (mmol/L)	1.34 (0.18)	1.25 (0.19)	1.25 (0.19)	1.28 (0.18)	1.12 (0.18)	1.12 (0.21)	1.24 (0.17)	1.23 (0.23)
S-ALP (U/L)	181 (79)	203 (90)	202 (92)	216 (91)	80 (37)	86 (32)	74 (29)	86 (41)*

6. DISCUSSION

6.1. Clinical significance and therapeutic implications

Osteoporosis and low BMD in children with motor disability

Our results show that a significant proportion of children with severe motor disability have subnormal bone mass and fractures and hence fulfill the diagnostic criteria for secondary osteoporosis. We found low (Z-score <-2.0) BMD or BMAD in 36% and 19%, of the 59 studied children with severe motor disability, respectively. The proportion of patients with low BMD has been even greater in some earlier studies. Henderson et al. (2002) reported that BMD Z-score in the distal femur was <-2.0 in up to 77% of subjects with moderate to severe CP. In another study involving children and adults with spastic quadriplegia, with an age range from 5 to 48 years, 58% had a Z-score <-2.0 (King et al. 2003). These discrepancies could be due to differences in the measurement site: in a group of children with moderate to severe CP more significant BMD reduction was observed in the distal femur relative to the spine (mean BMD Z-score -3.5 ±0.2 vs. -2.0±0.1) (Henderson et al. 2002). The proximal hip has been regarded as an unreliable BMD measurement site in children, and lumbar spine and whole body, excluding the head, are the preferred measurement sites (Gordon et al. 2008). In contrast to several earlier studies, we corrected all BMD measurements for bone size and bone age, and therefore, the results are likely to give a more accurate estimate of the proportion of subjects with subnormal BMD.

In our study, the median BMAD Z-score was significantly lower in patients with fractures (-1.7, range -5.0 – 0.0) than in those without fractures (-0.6, range -2.9 - +1.8) (P = 0.004). This is consistent with an earlier study on patients with severe CP: patients with a history of fracture had significantly lower lumbar spine BMD Z-scores than those without fractures, the mean BMD being -2.8 ± 0.3 and -2.1 ± 0.3, respectively (King et al. 2003). In a logistic regression model, low BMAD could be identified as a significant predictor for fractures, those with a BMAD Z-score < -1.5 having an almost 10-fold risk for fracture.

No earlier prevalence studies using the recently established criteria of childhood osteoporosis exist (Rauch et al. 2008). Based on BMD and fracture history, altogether 17% of the children with motor disability were diagnosed as having osteoporosis. This finding implies that children with motor disability comprise a major risk group for secondary osteoporosis.

All five children in the subgroup of neuromuscular disorders had a positive fracture history. Osteoporosis could be diagnosed in one boy with DMD and in one girl with SMA. In an earlier longitudinal study involving boys with DMD, BMD was subnormal already long before the boys lost their ability to move and spinal BMD Z-score further decreased from -0.8 to -1.7 and femoral BMD from -1.6 to -3.9 with loss of ambulation (Larson and Henderson 2000). The boy with DMD and osteoporosis in our cohort was able to walk despite having multiple spinal fractures. Impaired muscle strength and low muscle mass, in addition to bone loading, are likely to play a role in the maintenance of normal bone mass in muscular dystrophies. The complexity of bone formation and degradation and bone development is also reflected in a study involving children with MMC, which suggested that overall physical inactivity may have a systemic effect on bone formation, as BMD in children with MMC was not reduced only in the lower limbs but also in the distal radius (Quan et al.1998). Further, no differences were present between ambulants and nonambulants. Our study included 7 children with MMC, and osteoporosis was diagnosed in one of them.

No significant correlations were found between lumbar spine BMAD Z-score or fractures and S-25-OHD, P-PTH, and P-Pi levels, or calcium and vitamin D intakes. In children with fracture, the median P-Ca was higher than in those without fractures. The overall vitamin D levels were very low, and probably because of this uniformly suboptimal vitamin D status, we failed to show significance of vitamin D for fractures. We did not find any differences in vitamin D levels, lumbar BMAD Z-scores, or fracture rate between children with and without antiepileptic medication.

Fractures in children with motor disabilities

Altogether 34% (20/59) of the children had a history of one or several fractures. Fractures are common also in healthy children. According to a Swedish study, up to

42% of healthy boys and 27% of healthy girls sustain at least one fracture before the age of 17 years; the incidence peaks during puberty (Landin 1983). Here the fractures occurred evenly throughout childhood, with no evidence for a specific age of increased susceptibility. Further, lower limb fractures (70% of peripheral fractures) and spinal fractures predominated, and the fractures were often due to minimal trauma. This is consistent with earlier studies in children with motor disability. Previous studies have shown a fracture prevalence of 21-44% in these children (Larson and Henderson 2000, Henderson et al. 2002, McDonald et al. 2002). Lower limb fractures dominated, while most fractures in healthy children occur in the upper extremity (Landin 1983, Cheng and Shen 1993, Larson and Henderson 2000, McDonald et al. 2002). In a North American study, children with CP had a fracture rate of 4.0 per 100 per year (Stevenson et al. 2006), whereas the fracture rate in normal children in Scandinavia was <2.0 per 100 (Lyons et al. 2000). In children with spastic quadriplegia, the fracture rate did not differ between those with low and very low spinal BMD during a four-year follow-up, and low BMD in the lumbar spine did not predict fracture risk (Henderson 1997). In children with DMD, the peak of fracture incidence was reported to occur at 8–11 years (McDonald et al. 2002). Our findings are parallel to these observations. Fractures can be regarded as a sign of significantly impaired bone health, and several factors, including impaired weight-bearing, reduced muscle mass and strength, and potential hormonal disturbances, are likely to contribute to fractures in children with severe motor disability.

Vertebral fractures are a sign of abnormal bone quality. We found compression fractures in 25% of children, which is more than in other chronic conditions in children. Previous studies applying a similar methodology for vertebral fracture detection found compression fractures in 8% of children with renal transplantation, in 10% of steroid-treated children with juvenile idiopathic arthritis, in 20% of children with allogeneic stem cell transplantation, and in 18% of children with liver transplantation (Taskinen et al. 2007, Valta et al. 2007, 2008, 2009). Earlier studies of children with CP found no spinal compression fractures, which might be due to lack of systematic screening (Henderson 1997, Henderson et al. 2002, Stevenson et al. 2006, Mergler et al. 2009). Another study showed that DMD boys on long-term glucocorticoid treatment had a 32% incidence of vertebral compression fractures (King et al. 2007).

Bisphosphonates are increasingly used in children, but the indications and optimal dosing and treatment duration have not yet been established. According to a recent paper, a vertebral compression fracture should be regarded as an indication for bisphosphonate therapy (Bachrach and Ward 2009).

We found hypercalciuria to be an independent risk factor for fractures, with a 7-fold fracture risk. Hypercalciuria was found in 50% of those with fractures, compared with 15% in those without. In children with severe motor disabilities and low muscle mass, serum and urine creatinine levels may be low, and thus, U-Ca/U-Crea may not be an optimal measure for calcium excretion; hypercalciuria may reflect low muscle mass rather than increased calcium excretion. In our study, only 2/16 children with hypercalciuria had S-Crea under the age-specific reference value (11 μmol/L and 23 μmol/L). Since we did not measure whole-body DXA to evaluate body composition, we do not know the amount of muscle mass in these children. However, patients with fractures also had a higher S-Ca concentration than those without. These findings suggest that hypercalciuria may be secondary to immobilization, reflecting abnormal bone metabolism and impaired utilization of calcium.

Vitamin D deficiency and optimal vitamin D dosing

Based on our findings, vitamin D deficiency and insufficiency are alarmingly common in Finnish children with severe motor disabilities and adults with ID. Vitamin D deficiency (defined as <37.5 nmol/L in children and <50 nmol/L in adults) was present in 36% of children and 77% of adults. Altogether 95% of children and 98% of adults had S-25-OHD <80 nmol/L, which has been regarded as the optimal level for bone health.

Since the studied patient groups are at high risk of secondary osteoporosis, adequate vitamin D supplementation should be provided. Optimal dosing remains open, as several subjects in intervention studies failed to reach an optimal 25-OHD level. In adults with ID, a S-25-OHD concentration of >80 nmol/L was obtained by 42% of the PO group and by only 12% of the IM group. In children, only 19% reached an optimal S-25-OHD level. In the group of children with no supplementation, vitamin D levels

were even lower at the beginning of summer, indicating the need for vitamin D supplementation also during the summer months.

The observed low vitamin D concentrations are consistent with studies in healthy Finnish children, adolescents, and adults (Kauppinen-Mäkelin et al. 2001, Lamberg-Allardt et al. 2001, Lehtonen-Veromaa et al. 1999, 2002, Viljakainen et al. 2006, Välimäki 2007). Based on these observations, the national recommendations for vitamin D intake should be reconsidered. In general, there is an urgent need for studies on the optimal dosing, safety, and efficacy of long-term vitamin D supplementation. Recommendations concerning vitamin D supplementation are not always based on studies with modern techniques to assess S-25-OHD. Furthermore, earlier studies have considered the two different vitamin D preparations (D2 and D3) to be equal in efficacy and side-effects (Vieth 1999).

Some earlier studies have compared different vitamin D supplementation regimens in healthy populations and senior citizens (Heikinheimo et al. 1992, 1996, Guillemant et al. 2001, Trivedi et al. 2003, Diamond et al. 2005, Chel et al. 2008). In the elderly, a peroral dose of 100 000 IU D3 every four months was safe. This dose also decreased fracture risk in the study subjects (Trivedi et al. 2003). Annual intramuscular injections of 600 000 IU D3 were safe in vitamin D deficiency in the elderly (Diamond 2005). Heikinheimo et al. (1992, 1996) reported intramuscular injections of 150 000- 300 000 IU D2 to decrease risk for fracture. In adolescents, a peroral dose of 100 000 IU D3 three times every second month over the winter season was safe (Guillemant et al. 2001). Oral supplementation with 100 000 IU every fourth month was also effective and safe in correcting vitamin D insufficiency in people with ID (Vanlint and Nugent 2006).

In our study, peroral dosing was more efficient than intramuscular in correcting low S-25OHD values during a six-month observation; S-25OHD increased in the PO group on average 20 nmol/L more than in the IM group. Our study setting was probably not optimal, and based on S-25-OHD levels at six months, it is likely that the six-month interval between vitamin D injections is too long. We could not evaluate biochemical parameters at two to four months, and thus, were unable to analyze the peak vitamin D concentration obtained. The optimal dose interval therefore remains unknown. Further

studies are needed to establish optimal dosing, safety, and efficacy of long-term vitamin D supplementation in adults with ID.

IM injections may be a more convenient and possibly equally effective mode of treatment in adults with ID, who often have problems with swallowing. IM injections are also less expensive than oral vitamin D preparations (Diamond et al. 2005). Its major drawback in Finland is the lack of suitable preparations for wider clinical use.

Nutrition

Nutritional factors play an important role in acquisition of peak bone mass. In the present cohort, energy intakes, both sex- and age-specific, and intakes of some nutrients, especially vitamin D, were too low. The median energy intake was 76% of the recommendation and less than 80% of the recommendation in 57% of children. The median vitamin D intake was 76% of the recommendation, and serum 25-OH-vitamin D concentrations were low, the median being 46 nmol/L.

Malnutrition predisposes a child to disturbances in immune functions and subsequently to infections (Seddon and Khan 2003), which may be an important clinical issue in this patient population. In addition, malnutrition decreases muscle strength (Russell et al. 1983). This may further impair motor function, moving ability, and coordination and reduce the effectiveness of coughing, predisposing to aspiration.

Better nutritional status, as assessed by triceps skin-fold thickness or weight z-scores, has been associated with better BMD in children with moderate to severe CP (Henderson et al. 2004, 2005). Since low BMD and secondary osteoporosis are frequent features in children with severe motor disabilities, every effort should be taken to increase PBM and prevent additional disability caused by fractures. Because there are no official guidelines for these children, feeding problems should be assessed individually.

Low milk intake in childhood and adolescence is associated with decreased bone mass and greater risk of fracture in adulthood (Kalkwarf et al. 2003). Studies suggest that increased use of dairy products is more effective in improving bone health than calcium

supplementation (Cheng et al. 2005). Calcium supplementation without vitamin D seems to have no effect on BMD in children (Winzenberg et al. 2006). In contrast to observations by Bertoli et al. (2006b), we found that calcium intake of the Finnish children participating here was fairly good because of the national tradition of consuming abundant dairy products. The median calcium intake was 142% of the recommendation. All children, except one percutaneous endoscopic gastrostomy tube (PEG)-fed child, used dairy products daily. This high calcium intake was probably protective against secondary hyperparathyroidism despite low serum vitamin D concentrations (Steingrimsdottir et al. 2005).

While vitamin A and phosphorus intakes were high, their serum concentrations were within the normal range. Dairy products are the main source of phosphate. Both high phosphate intakes and high phosphate serum concentrations have been associated with adverse skeletal and cardiovascular effects (Tonelli et al. 2005, Kemi et al. 2006). Excessive vitamin A intake has been associated with osteoporosis, but the findings are controversial (Michaelsson et al. 2003).

Although parents reported feeding difficulties in 11 children (20%), only two children were fed by PEG. While PEG improves the caregivers' quality of life, its benefits on the child's growth are controversial; there is a risk of overfeeding, and thus, for excessive fat mass accumulation (Sullivan et al. 2004, 2006). However, many children in the present cohort might have benefited from PEG feeding.

Clinical message

Prevention of osteoporosis in children with severe motor disability should include adequate intakes of vitamin D, calcium, and energy. All children with severe motor disability and individuals with ID living in an institution should be supplemented with a minimum of 800 IU (20 µg) of vitamin D3 daily. The target S-25-OHD should be >80 nmol/L which has been considered optimal for bone health. In feeding problems, adequate energy supplies should be individually assessed with the help of a dietitian. PEG feeding should be encouraged if optimal energy and nutrient intakes fail to be achieved by oral feeding.

Since osteoporosis and fractures are prevalent in children with severe motor disability, efforts should be made to enhance bone mass gain. According to previous studies, BMD can be improved also in people with motor disability by increasing weight-bearing activity (Caulton et al. 2004). Short spurts of repetitive muscular contractions, which according to the mechanostat theory (Schoenau 2005) are beneficial for bone mass gain, are not always possible for people with severe motor disability. The impact and use of low-magnitude, high-frequency mechanical signals (Ward et al. 2004, Gilsanz et al. 2006, Rubin et al. 2006) as well as other means to improve BMD by physical activity should be studied further in this patient population.

Compression fractures were common in children with severe motor disability. However, the cross-sectional nature of the study does not allow us to predict the significance of these fractures or whether all children with severe motor disability should be actively screened for compression fractures. Similarly, although low BMD was a common finding, its ability to predict future fractures cannot be estimated in a cross-sectional setting. While prospective studies and screening guidelines are awaited, emphasis should be placed on active prophylaxis of osteoporosis and fractures.

Bone health in chronically ill children is a fairly new research area within pediatrics and pediatric neurology. Now that the high prevalence of osteoporosis and vitamin D deficiency has been recognized as a significant medical problem in these children, more prospective studies with larger study populations are needed to establish optimal treatment and guidelines for screening and follow-up.

6.2. Limitations of the study

The studies suffer from some limitations. A major limitation is the small sample sizes in Studies **I** and **II**. Further, the cohort is heterogeneous with regard to etiology of the disability. However, the study group represents a normal outpatient population in any pediatric neurology department in Finland. Thus, our observations are relevant to pediatric neurologists and can be extended to the patient population in their clinics.

The interpretation of pediatric DXA is influenced by growth and developmental stage of the child, and if these are not taken into account, osteoporosis can be misdiagnosed. A BMD obtained by DXA is dependent on bone size. DXA underestimates BMD in a short child with small bones and overestimates BMD in a tall child with larger bones. Chronic diseases often influence the growth and development of a child; usually a child with a chronic condition is small for his/her age. We compared the measurements with age- and sex-specific reference values. These were further adjusted for bone size by a mathematical model and for pubertal stage by using bone age instead of calendar age when these were discrepant. Despite these efforts, the DXA-derived BMD values may not be completely reliable in the assessment of bone health in children with motor disability. Furthermore, DXA fails to provide information on bone quality, which might not be parallel to bone mass and is known to influence fracture resistance.

Most DXA measurement sites lack pediatric reference data, and published data are specific for the equipment and the manufacturer; DXA results measured with different equipment are therefore not comparable. We used previously published normative data based on the measurements of 444 healthy Caucasian children in the Netherlands using the same equipment. This was regarded as more reliable than the published Finnish reference values for the equipment (Kröger et al. 1993), as these were based on a very small number of subjects (N=65) and the variation was large.

The association between fractures and low BMD in children is not as clear as in adults, and DXA may not be an optimal means of measuring bone strength. However, our results showed a clear association between low BMAD Z-score and fracture risk.

The weakness of the food diaries filled by parents is that the evaluation depends completely on the information given by them. A three-day food diary is, however, a widely accepted standard method to evaluate nutrient intakes (Nelson et al. 1989), and calculations were made by two registered dietitians. Because energy needs may differ between children with severe motor disability and healthy children, the study would have greatly benefited from an evaluation of energy expenditure (Stallings et al. 1996), but such a method was not available for the study.

Height measurements are complicated in children with motor disabilities because of contractures and scoliosis. The obtained heights can be underestimations, and thus, true height-adjusted weights and BMIs may be even lower than those reported here.

The two vitamin D interventions left several questions unanswered. In Study **III**, a longer intervention time would have provided an opportunity to detect more marked changes in bone metabolism, or changes in BMD and muscle strength. However, the study was primarily designed to determine optimal vitamin D dosing and can thus be regarded as a pilot study. Our observations provide information regarding vitamin D dosing to be used in subsequent intervention studies assessing more complex skeletal and health-related parameters in children with motor disabilities. In **Study IV**, the timing of the second blood samples was not optimal for detecting the peak concentration of S-25-OHD; the optimal dosing and interval for intramuscular vitamin D remains open.

6.3. Future perspectives

Low BMD, a high fracture rate, and low vitamin D levels were observed in the children studied. Vitamin D supplementation clearly should be used. However, the dosing remains open, as does other means to decrease the risk of fractures and low BMD in these children. The optimal timing and dosing of intramuscular vitamin D injection requires further research.

We found hypercalciuria to be a clear risk for fractures in children with severe motor disabilities. The clinical implications of this finding remain to be elucidated in future studies.

Improvement of nutritional status in children with motor disability may bring significant health benefits. Methods to define, assess, and achieve optimal nutritional status in children with motor disabilities remain unknown and should be addressed in future research.

Bisphosphonates have been used in the treatment of osteoporosis in childhood. Knowledge of their effects in children is based mainly on studies carried out in children with OI. The potential benefits of bisphosphonates in the treatment of secondary osteoporosis in children with motor disabilities await elucidation (Bachrach and Ward 2009).

7. SUMMARY AND CONCLUSIONS

This study demonstrated that low BMD and vertebral compression fractures are common in children with severe motor disabilities. Low BMD (Z-score <-2.0) was observed in more than half of the children. Compression fractures were found in 25% of the children. Further, according to recent diagnostic criteria, childhood osteoporosis was diagnosed in 10 of the 59 children with severe motor disability. Low BMD and hypercalciuria were significant risk factors for fractures.

Compromised weight-bearing activity is a clear risk factor for low BMD. Other risk factors, such as poor nutrition and vitamin D insufficiency, were commonly observed in children with severe motor disability. Moreover, adults with ID living in an institution had poor vitamin D status. Only 1% of adults with ID and 5% of children with motor disability had a S-25-OHD level above 80 nmol/L.

Vitamin D deficiency and poor nutrition are risk factors that can be treated. Both patient groups should be provided with adequate calories in the diet and vitamin D supplements to prevent osteoporosis. Peroral vitamin D supplementation of 800-1000 IU/day (20-25 µg/day) was safe and efficient, and should be used in these patients all year round. Evaluation and prevention of osteoporosis are recommended in the follow-up of all children with severe motor disabilities and patients with ID.

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