Bone mineral density in pediatric inflammatory bowel disease

Susanne Schmidt

University of Gothenburg

Gothenburg 2010
Department of Pediatrics
The Institute of Clinical Sciences, Sahlgrenska Academy
University of Gothenburg, Sweden
Contents

ABSTRACT .......................................................................................................................... 1
LIST OF PAPERS .................................................................................................................... 3
ABBREVIATIONS .................................................................................................................. 5
INTRODUCTION ..................................................................................................................... 7
THEORETICAL BACKGROUND ............................................................................................. 9

- Inflammatory bowel disease 9
  - Definition 9
  - Epidemiology 9
  - Etiology 10
  - Disease presentation 10
  - Diagnostic criteria 11
  - Treatment 11
- Bone physiology 12
  - Remodeling: activation phase 12
  - Remodeling: resorption phase 13
  - Remodeling: reverse phase 13
  - Remodeling: formation phase 13
  - Cortical and trabecular bone 14
  - Modeling 14
  - Growth plate 15
- Peak bone mass 16
- Osteoporosis: significance, risk factors and definitions 18
  - Definitions in adults 19
  - Definitions in children 19
- Clinical investigations of BMD 20
  - Dual-energy X-ray absorptiometry 20
  - Peripheral Quantitative Computed Tomography 21
  - Quantitative ultrasound 21
  - Biochemical markers of bone metabolism 22
- Decreased BMD in IBD patients 22
  - Adult IBD patients 23
  - Pediatric IBD patients 24
- Pathogenesis of decreased BMD in IBD 25
  - Tumor necrosis factor-α 26
  - RANKL-OPG system 27
  - Corticosteroids 27
  - Vitamin D metabolism 28
  - Parathyroid hormone 29
Abstract

Low bone mineral density (BMD) has been highlighted as a potential problem in children with inflammatory bowel disease (IBD), which is one of the most common chronic childhood diseases in the westernized world. The mechanisms behind reduced BMD in pediatric IBD are still not completely understood, but several factors that influence bone mineralization have been discussed. These include the chronic inflammation itself, which causes the release of cytokines from the inflamed bowel, treatment with corticosteroids, low body weight, limited physical activity, vitamin D deficiency and genetics. Decreased peak bone mass in young adulthood may predispose for the development of osteoporosis later in life and this in turn may lead to osteoporosis-related fractures.

The aim of this thesis was to investigate BMD, body composition and growth in a population of Swedish children and adolescents with IBD over a two-year period. A second objective was to study the familial resemblance of BMD in pediatric IBD patients.

The thesis was designed as a prospective, longitudinal, population-based project with patients from two pediatric centres in Western Sweden (Göteborg and Borås). In order to evaluate BMD and body composition the patients and their parents underwent dual-energy X-ray absorptiometry (DXA) at the time of inclusion in the study. Two years later the DXA measurement was repeated in the IBD patients. Additionally, clinical data, body weight, height, Tanner stage, bone age and blood samples for various hormone analyses were obtained. Age at peak height velocity (PHV) was calculated using special software.

Low bone mass was found to be prevalent in this population of Swedish pediatric patients with IBD both at baseline and at follow-up two years later. Possible risk factors for lower BMD are male gender, low BMI and treatment with azathioprine, which is a likely marker of disease course severity. However, the data indicate that both males and females have the potential to recover BMD into early adulthood. Furthermore, this study demonstrated that, regardless of the presence of a chronic inflammatory condition, the BMD of children and adolescents with IBD is significantly related to that of their parents. Normal vitamin D levels were present in the group of pediatric IBD patients and showed a significant seasonal variation with lower levels during winter time. No significant correlation was found between vitamin D levels and BMD. Elevated levels of intact parathyroid hormone (iPTH) were seen in the patients under 16 years of age despite normal vitamin D levels. Vitamin D and iPTH levels were inversely related. Lean mass deficits were present in the oldest age groups and were most pronounced in males and those with Crohn’s disease. Age at PHV was significantly delayed by around one year in both females and males and this may indicate suboptimal growth.

The data from this thesis support the conclusion that pediatric patients with IBD should be evaluated with DXA at some point during the course of their disease, if possible soon after being diagnosed.
Abstract

Key words: bone mineral density, inflammatory bowel disease, children, Crohn’s disease, ulcerative colitis, vitamin D, parathyroid hormone, familial resemblance, body composition, growth
List of Papers

I Schmidt S, Mellström D, Norjavaara E, Sundh SV, Saalman R. *Low Bone Mineral Density in Children and Adolescents with Inflammatory Bowel Disease: A Population-Based Study from Western Sweden*. Inflamm Bowel Dis 2009; 15 (12): 1844-50

II Schmidt S, Mellström D, Norjavaara E, Sundh V, Saalman R. *Familial Resemblance of Bone Mineral Density in Children with Inflammatory Bowel Disease*. In press (Journal of Pediatric Gastroenterology and Nutrition)

III Schmidt S, Mellström D, Norjavaara E, Sundh V, Saalman R. *Longitudinal Assessment of Bone Mineral Density in a Population of Children and Adolescents with Inflammatory Bowel Disease*. Submitted

IV Schmidt S, Mellström D, Norjavaara E, Nilsson S, Saalman R. *Body Composition, Growth and Puberty in Children and Adolescents with Inflammatory Bowel Disease*. In manuscript
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>alkaline phosphatase</td>
</tr>
<tr>
<td>BMD</td>
<td>bone mineral density</td>
</tr>
<tr>
<td>aBMD</td>
<td>areal bone mineral density</td>
</tr>
<tr>
<td>vBMD</td>
<td>volumetric bone mineral density</td>
</tr>
<tr>
<td>BMC</td>
<td>bone mineral content</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CD</td>
<td>Crohn's disease</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>DXA</td>
<td>dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>GH</td>
<td>growth hormone</td>
</tr>
<tr>
<td>IBD</td>
<td>inflammatory bowel disease</td>
</tr>
<tr>
<td>IC</td>
<td>indeterminated colitis</td>
</tr>
<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>M-CSF</td>
<td>macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>MP</td>
<td>mercaptopurine</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor kappa beta</td>
</tr>
<tr>
<td>OPG</td>
<td>osteoprotegerin</td>
</tr>
<tr>
<td>PCDAI</td>
<td>pediatric Crohn disease activity index</td>
</tr>
<tr>
<td>PHV</td>
<td>peak height velocity</td>
</tr>
<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
</tr>
<tr>
<td>pQCT</td>
<td>peripheral quantitative computed tomography</td>
</tr>
<tr>
<td>QUS</td>
<td>quantitative ultrasound</td>
</tr>
<tr>
<td>RANK(L)</td>
<td>receptor activator of NF-κB (ligand)</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>UC</td>
<td>ulcerative colitis</td>
</tr>
</tbody>
</table>
Introduction

Low bone mineral density (BMD) has been recognized as a potential health problem in both adults (1-6) and children (7-13) suffering from inflammatory bowel disease (IBD). The occurrence of low BMD has shown great variation in pediatric studies from different countries, ranging from 5 to 70%. Recently, decreased BMD has been reported even in relation to other chronic diseases that begin in childhood, such as insulin-dependent diabetes mellitus or cystic fibrosis (14-18).

IBD is one of the most common chronic pediatric diseases in the Western world and its incidence is increasing (19-21). Up to 25% of all patients with IBD develop this condition during childhood or adolescence (22).

The pathogenesis of disturbed bone mineralization in pediatric IBD is considered to be multifactorial. Factors include cytokines released from the inflamed bowel due to the chronic inflammation (23), treatment with corticosteroids (24), low body weight (25), vitamin D deficiency (26) and genetics (27).

In healthy individuals, BMD increases rapidly during puberty and reaches its highest level, peak bone mass, in the lumbar spine at about the age of 16 in females and about the age of 20 in males (28). During development, BMD may be affected by disturbances such as inflammatory conditions. Decreased peak bone mass in young adulthood may predispose for the development of osteoporosis later in life and this in turn may lead to osteoporosis-related fractures (29).
Theoretical Background

Inflammatory bowel disease

Definition

The term inflammatory bowel disease describes a chronic (lifelong) and relapsing intestinal inflammation. IBD may affect different parts of the gastrointestinal tract and it has two major forms: Crohn’s disease (CD), which can attack any part of the gastrointestinal tract, and ulcerative colitis (UC), which is limited to the rectum and colon. The feature of CD is a discontinuous inflammation that affects all layers of the gastrointestinal wall, whereas UC is characterized by a continuous inflammation that is limited to the mucosa.

Epidemiology

IBD is a disease that has a peak incidence during the 2nd and 3rd decades of life. Up to 25% of all patients with IBD develop the disease during childhood and adolescence (22). IBD is considered one of the most common chronic childhood diseases in the Western world; in Sweden alone, over 200 children below 16 years of age are diagnosed with IBD every year. Epidemiological studies from Sweden show an increasing incidence of pediatric IBD, especially for CD (19). While the incidence of overall IBD between 1984 and 1986 was 4.6 per 100,000 children below 16 years of age, the figures rose during the following decade to 7.0 per 100,000 (30). Between 2002 and 2007 the incidence was even higher at 11.5 per 100,000 children (21).
Theoretical Background

Etiology

The etiology of IBD is still unknown but it is considered to be multifactorial. Recently, it has been hypothesized that a currently unidentified triggering event may, in a genetically susceptible individual with an altered intestinal microbial flora and with particular environmental factors, activate an aberrant immune response, which results in a chronic intestinal inflammation (31). Cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) that are released from the inflamed mucosa are key players in this inflammatory process. The hygiene hypothesis has been posited as an explanation for the increasing incidence of IBD. This was formulated in 1989 by David Strachan in order to explain the increasing incidence of allergy in parts of Europe during the 20th century (32). He postulated that microbial stimulation early in childhood is necessary in order for normal functioning of the immune system to develop. This assumption has later been applied to IBD (33, 34).

Disease presentation

Major symptoms that raise suspicion of IBD are diarrhea, bloody stools, abdominal pain, anorexia, weight loss and/or fever. Specific symptoms of pediatric IBD are growth retardation and delayed puberty (35). In contrast to adult IBD, pediatric IBD seems to have a more aggressive course and a more extensive distribution (36). The involvement of the colon is more common for pediatric CD patients than for adults, and in up to 80% of the pediatric patients with UC pancolitis is present at the onset of the disease (37).
Diagnostic criteria

IBD is diagnosed on the basis of clinical, radiological, endoscopic and histological findings. Diagnostic criteria used widely in pediatric gastroenterology are provided by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (38). However, in about 15% of the pediatric patients a clear diagnosis cannot be established and the colitis is therefore classified according to the Montreal criteria as “inflammatory bowel disease, type unclassified” (IBDU) (39). The term indeterminate colitis (IC) used to be used, but this term is now only recommended once colectomy with complete histological examination has shown that a definitive diagnosis cannot be established (40).

Treatment

Treatment of IBD is based on pharmacological, nutritional and / or surgical interventions. In Sweden almost all children and adolescents with IBD are treated according to the national guidelines of the Gastroenterological Working Group of the Swedish Pediatric Society (www.blf.net). These guidelines are similar to the European recommendations (41). Regarding pharmacological treatment, corticosteroids play still an important role for inducing remission in active IBD. Early introduction of immunomodulators (e.g. azathioprine) is recommended as maintenance therapy in disease with frequent relapses. Infliximab, a monoclonal antibody to the proinflammatory cytokine TNF-α, can be used in severe cases. In pediatric patients, one of the main challenges is reducing the severity of intestinal inflammation in order to optimize growth and pubertal development (42).
Theoretical Background

Bone physiology

Not only is bone the human body’s most important store of calcium, phosphate and magnesium but it is also a living tissue with an active metabolism. The major process that takes place in the adult skeleton is that of remodeling. Remodeling occurs throughout life in the bone multicellular unit in order to maintain a constant bone volume and calcium homeostasis. This involves resorption of the bone by osteoclasts and bone formation by osteoblasts in a physiologically coupled process (43). Remodeling also takes place in growing children, but the main process in the growing skeleton is modeling. Modeling occurs when the physiologically coupled process of bone resorption and bone formation is uncoupled such that bone formation predominates. Since the process of remodeling involves both bone resorption and bone formation it is clarified below before details of modeling and the growth plate are presented.

Remodeling: activation phase

Quiescent osteoblasts are activated by several factors, such as micro-fractures, alterations in mechanical loading or released 1,25-hydroxyvitamin D, parathyroid hormone (PTH) (44), prostaglandin E2 (PGE2), insulin-like growth factor 1 (IGF-1) or TNF-α (45). In turn, the activated osteoblasts increase their expression of receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL). RANKL binds to the receptor activator of NF-κB (RANK), which is expressed by osteoclast progenitor cells. These progenitor cells differentiate subsequently into osteoclasts. Furthermore, the osteoblasts produce macrophage colony-stimulating factor (M-CSF), which is another essential factor for osteoclast differentiation, and osteoprotegerin (OPG). OPG is a decoy receptor that competes with RANK for RANKL (46). Thus, it
Theoretical Background

is the balance between RANKL, as a stimulator of osteoclastogenesis, and OPG, as an inhibitor, that determines the amount of resorbed bone (47). Another suppressor of RANKL is estrogen (48), which explains the increased rate of bone resorption and osteoporosis in postmenopausal women. Furthermore, inflammatory cytokines act either directly on osteoclasts or indirectly by regulating RANKL and OPG expression (49). It has been found that TNF-α has synergistic effects with RANKL (50). The RANKL-OPG system can also be triggered by activated T cells which play a central role in IBD and this establishes the missing link between bone metabolism and autoimmune disease (51).

Remodeling: resorption phase

After differentiation the osteoclasts polarize, adhere to the bone and dissolve it through acidification and release of enzymes, such as cathepsins K (52). When the osteoclasts have fulfilled their role, they undergo apoptosis.

Remodeling: reverse phase

This phase is not completely understood, but it is probably characterized by removal of debris from matrix degradation that is produced during the resorption phase. The involved cell types are reverse cells, which are macrophage-like cells (53).

Remodeling: formation phase

Several growth factors, including bone morphogenetic proteins (BMPs) (54), fibroblast growth factors (FGFs) and transforming growth factor β (TGF-β), lead to the recruitment of osteoblasts which, as the principal bone-forming cells, start to produce new
bone matrix. The process of bone formation proceeds in two steps: firstly, the formation of extracellular matrix followed by mineralization. The extracellular matrix, also called osteoid, consists mainly of collagen type 1 (90%), but also of non-collagen proteins such as osteocalcin, osteonectin or osteopontin. Secondly, the mineralization starts with the precipitation of hydroxyapatite crystals, which grow over the course of the following year. It is largely unknown how these crystals precipitate but alkaline phosphatase (ALP) released from the osteoblasts are assumed to play an important role in this process (55).

Cortical and trabecular bone

Osteoblasts can form two different types of bone: cortical and trabecular bone. Cortical (dense or compact) bone is quite dense with a porosity of between 5% and 10%. This bone type is found in the shaft of long bones and forms the outer shell around trabecular bone at the end of joints and the vertebrae. Trabecular bone (also known as cancellous or spongy bone) is much more porous with a porosity of between 50% and 90%; it is found in the ends of long bones, in vertebrae and in flat bones like the pelvis. The skeleton is made up of 80% cortical and 20% trabecular bone. Due to its porosity trabecular bone has a large surface area. The metabolic activity of trabecular bone is eight times higher than in cortical bone. Trabecular bone is therefore more sensitive to disturbances in bone formation or bone resorption. Osteoporosis-induced fractures occur mainly in trabecular bone.

Modeling

Under certain circumstances, the process of modeling may occur. In this case, the coupling phenomenon is reversed - bone formation may take place without preceding bone resorption and
bone resorption may take place without subsequent bone formation. During childhood and adolescence, bone formation dominates until peak bone mass (maximum bone mass) is reached at the end of the second decade of life.

Growth plate

The growth plate (or epiphyseal plate) is a cartilage structure found at each end of a long bone. It is responsible for the longitudinal growth of bones and shows three typical layers: resting, proliferative and hypertrophic zone (56, 57). Briefly, in these zones chondrocytes synthesize cartilage that is subsequently ossified in a process called enchondral ossification. Chondrocyte regulation is complex and involves both paracrine (PTH-related peptide (PTHrP), Indian Hedgehog (Ihh), vascular endothelial growth factor (VEGF)) and endocrine factors (growth hormone (GH), IGF-1, corticosteroids, thyroid hormone, estrogen, androgen, vitamin D).

A decline in growth rate is due to a mechanism that is intrinsic to the growth plate, called senescence (aging) (58). Senescence leads to decreased growth and finally to a fusion of the epiphysis. Growth plate senescence is probably not a function of age per se but a function of the number of cell replications of growth plate chondrocytes. Thus, growth will slow down as the proliferative capacity of the chondrocytes becomes exhausted. Estrogen plays an important role in the senescence of the growth plate and it therefore promotes epiphyseal fusion (59).

Long bones increase not only in length but also in width. This process is called intramembranous bone formation and occurs at the periosteal surface of the bone. It is combined with bone
resorption at the endosteal surface and this enlarges the medullary cavity.

**Peak bone mass**

The maximum bone mass achieved by the end of skeletal maturation is called *peak bone mass*. During subsequent decades, bone mass remains constant until bone resorption begins to predominate in women after menopause and, later, also in men. As a consequence, bone mass decreases and the risk of fracture increases. It is widely accepted that peak bone mass is the strongest predictor of the future risk of osteoporosis (60).

Gender differences in bone mass develop after the onset of puberty. This appears to be the result of a greater increase in bone size in males than in females and this is associated with greater increase in cortical thickness (61). In contrast, volumetric trabecular density shows no variation according to gender (62).

According to long-established thinking, peak bone mass is believed to be reached at any skeletal site in both sexes in the mid-thirties. However, there is no evidence that supports this hypothesis (28). On the contrary, it has been observed that females achieve peak bone mass in the femoral neck and lumbar spine before the end of the second decade (63). In males, peak bone mass has been shown to be attained in Swedish men by 18 to 20 years of age in the spine and femoral neck, but not in the radius or tibia (64). This suggests that the age of attainment of peak bone mass is site-specific and that a phase of consolidation continues even after the second decade in males.

Many factors are considered to influence bone mass accumulation during growth: genetics, gender, dietary components (such as
Theoretical Background

proteins and calcium), endocrine factors (sex hormones (65), vitamin D (66, 67), IGF-1 (68)) and mechanical forces (physical activity (69), body weight). Factors such as genetics and gender are non-modifiable, whereas nutrition or physical activity are considered to be modifiable factors.

Genetics is one of the most important factors for the achievement of peak bone mass. It is estimated that around 75% of the variance in bone mass in a population is determined by genetic factors, as demonstrated in twin and family studies (70-75). Several possible candidate genes have been evaluated, such as the vitamin D receptor gene, the estrogen receptor gene, the collagen-1-alpha-1 gene (76, 77), the genes for growth hormone and its receptors, and / or those for cytokines involved in bone metabolism. There does not appear to be one single gene that determines peak bone mass but there is consensus that the heredity of peak bone mass is polygenic in nature (29).

Since genetic factors account for up to 75% of the variance in BMD, it has been held that the remainder could be accounted for by modifiable factors. It has therefore been claimed that optimization of peak bone mass through calcium supplementation or physical activity during childhood and adolescence may have long-lasting effects and even prevent fractures in adulthood (78). However, there is growing evidence that bone does not, in fact, work like a bank account in which deposits may be made during childhood and withdrawals during adulthood (79). Gafni and co-workers demonstrated in a rabbit model that treatment with high doses of glucocorticoid during the growth period had little effect on adult bone mass (80). Studies have shown that physical activity has a positive effect on BMD. However, this effect does not persist after the cessation of training (81, 82). It has also been
shown that calcium supplementation is beneficial for bone mass acquisition (83), but there is doubt as to whether this effect persists when supplements have been discontinued (84). These findings indicate that bone is a homeostatic system which, after any disturbance, resumes a set point that appears to depend on recent events but not on those from the distant past (79).

**Osteoporosis: significance, risk factors and definitions**

Osteoporosis is considered to be one of the most common metabolic bone diseases and a worldwide health problem. According to the World Health Organization (WHO) osteoporosis is regarded a "... progressive systemic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture ..." (85). The WHO has predicted that the number of hip fractures caused by osteoporosis worldwide will rise from 1.7 million in 1990 to 6.3 million in 2050 (86).

Two forms of osteoporosis may be distinguished. Primary osteoporosis is caused by biological aging, menopause and lifestyle factors such as diet, physical inactivity, smoking and alcohol. Secondary osteoporosis is caused by several diseases, such as hyperthyreotoxicosis, IBD, rheumatoid arthritis, or by drug treatment, such as with corticosteroids. The United States National Institutes of Health have identified risk factors associated with low BMD in adults (60), including female gender, higher age, estrogen deficiency, white race, family history of osteoporosis, smoking, history of fracture, low weight and low body mass index (BMI).
Individuals with osteoporosis have an increased risk of fractures compared to controls. Fracture risk increased in adult women by a factor of 1.5 to 3 for every standard deviation (SD) reduction in BMD (87). In a cohort of young girls, a twofold increase in fracture risk for every SD reduction in BMD was demonstrated (88).

Definitions in adults

Furthermore, the WHO has proposed expressing the measured absolute value of BMD as the so-called T-score. This is defined as the number of SDs by which a given BMD measurement differs from the mean BMD of young, healthy adults. A T-score < - 1.0 SD but > - 2.5 SD is considered to be osteopenia and < - 2.5 SD qualifies as osteoporosis (85). T-score < - 2.5 SD in combination with a prevalent vertebral fracture is referred to as manifest osteoporosis. It should, however, be noted that the T-score should only be used in postmenopausal women and men aged 50 or older. In all other adult and especially pediatric patients Z-scores should be used instead; these refer to age and gender-matched reference values. The Z-score is defined as the number of SDs by which a given BMD measurement differs from the mean BMD of healthy individuals of the same gender and age.

Definitions in children

According to the recommendations of the International Society for Clinical Densitometry (ISCD) from 2007 (89), the diagnosis of osteoporosis in children and adolescents (males and females ages 5-19 years) should not be made on the basis of densitometric criteria alone. The diagnosis in the pediatric group requires the presence of both a clinically significant fracture history and low BMD or bone mineral content (BMC). A clinically significant fracture history includes at least one of the following:
long bone fracture of the lower extremities, vertebral compression fracture, or and two or more long-bone fractures of the upper extremities. Low BMD or BMC is defined as an areal BMD or BMC Z-score (as described above) that is less than or equal to -2.0, adjusted for age, gender and body size, as appropriate. The term osteopenia should not be used for those in the pediatric group.

Clinical investigations of BMD

Dual-energy X-ray absorptiometry

Since the late 1980s dual-energy X-ray absorptiometry (DXA) has become the most readily available, non-invasive clinical technique for investigating BMD. It is widely used in adult medicine as the current gold standard. Its use in pediatrics is rising rapidly as bone health in children is becoming an area of growing concern.

The principle of DXA is based on the transmission of X-rays through the body at high and low energy levels (90). The fact that these energy levels become attenuated differently by soft tissue and bone means that the method enables these two tissue types to be distinguished. This technique therefore not only provides information about the bone but also about the soft tissues or body composition. Since the ionizing radiation dose that is used is relatively low, at only a tenth of that of a chest radiograph, and since the scan time is less than five minutes, this technique is suitable for examining children. Reproducibility with small variability and relatively low costs are other advantages.

DXA is a two-dimensional technique for measuring BMD. It does not measure true density but assesses the areal BMD (aBMD), which, in most studies, is referred to simply as BMD. Different skeletal regions of interest may be assessed: the whole body, the
lumbar spine, the proximal femur and the radius. The absolute values of BMD (g/cm$^2$) that are obtained are expressed as T-scores or Z-scores (as described above in the chapter “Osteoporosis”).

One of the limitations of DXA is that it does not reflect real density but the ratio of bone mineral content over an area (90). This may result in underestimation of BMD in pediatric subjects with growth retardation, such as some of our IBD patients. It is possible to compensate for these inaccuracies by correcting BMD values for bone age. Herzog and co-workers (13) showed that the figure for the patients with abnormal BMD Z-score < -2.0 SD fell from 44% to 26-30% when this adjustment was made. In other studies aBMD was mathematically transformed into volumetric BMD (vBMD) (11). Another limitation of DXA is that it cannot make distinctions between cortical and trabecular bone.

Peripheral Quantitative Computed Tomography

Peripheral Quantitative Computed Tomography (pQCT) enables measurement of the “true” vBMD (91). Sites of measurement are the radius, the tibia and the femur. Furthermore, pQCT distinguishes between cortical and trabecular bone. This procedure has so far only been put to limited use for pediatric patients.

Quantitative ultrasound

Quantitative ultrasound (QUS) is based on the attenuation of the ultrasound beam when it passes through the investigated region of interest (91). It is only possible to measure the peripheral skeleton at sites such as the calcaneus, the tibia or the radius. Since QUS does not use ionizing radiation, it is especially
appropriate for examining children. Pediatric studies of both healthy and diseased children have been carried out to assess the method. A study of pediatric patients with CD showed that by comparison with the DXA method, the investigation of the radius and tibia with QUS was not sufficiently sensitive to detect lower BMD (92). QUS should therefore only be used as a complement in clinical practice.

Biochemical markers of bone metabolism

Several markers of bone formation and bone resorption can be identified (93), such as markers of osteoblast activity (e.g. osteocalcin, bone ALP), osteoclast activity (e.g. tartrate-resistant acid phosphatase), various collagen propeptides (e.g. amino-terminal and carboxy-terminal propeptides of type 1 procollagen) and other breakdown products (e.g. hydroxyproline) (94).

Some pediatric studies have investigated their application to screening for metabolic bone disease in IBD (10, 95). To my knowledge, with the exception of bone ALP, these markers currently have only limited use in clinical routine.

Decreased BMD in IBD patients

The association between IBD and BMD was first described in 1964 by Edwards and Truelove (96). They reported a low prevalence of osteoporosis in UC patients (1.4%), according to conventional radiological signs of osteoporosis and fractures. Since the introduction of new methods of BMD measurement, particularly DXA in the early 1990s, several studies have concluded that low BMD is a common problem in both adults (1-6) and children (7-13) who suffer from IBD.
Adult IBD patients

The reported prevalence of osteopenia in adult IBD patients varies between 16% and 62% in cross-sectional studies (3, 4, 6). Osteoporosis was shown to be present in 5% to 38% of the patients (3, 4, 97). Whereas some authors have reported a significant difference in BMD between CD and UC (5, 98) others have not found this BMD difference (2, 3). Interestingly, Ghosh et al. (5) showed that even newly diagnosed patients with CD had significantly lower BMD than those who had recently been diagnosed with UC. Other investigations focused only on CD (6, 25) or on UC patients (99, 100).

A population-based two-year follow-up study from Norway (101) found that BMD mean Z-scores were significantly lower in CD patients than in UC patients and healthy controls. No significant changes in BMD occurred among either CD or UC patients during follow-up. In another longitudinal study by Dinca (3), BMD was found to be low but stable over time in CD patients. By contrast, the UC patients in this study showed significant decrease in BMD during follow-up and this was negatively correlated with the use of corticosteroids. This significant negative correlation between BMD and steroid intake has also been noted by others (97, 99). Onset of IBD < 18 years of age (6), low body weight (25) and low BMI (4) have been discussed as other predictive factors for disturbed bone mineralization. Gender, disease duration or disease localization do not seem to be correlated with low BMD (4).

Additionally, it has been observed that low BMD may improve after surgery (100) and that patients in remission have higher BMD than those with active disease (5, 102). These findings emphasize the importance of proinflammatory cytokines that are released from the inflamed gut in the pathogenesis of
osteoporosis in IBD patients. A recent report has indicated that maintenance therapy with anti TNF-α antibodies (infliximab) is associated with improved BMD in CD patients (103).

Only a few studies have investigated the clinical relevance of low bone mass in IBD patients. In a prospective study of 293 patients with CD, manifest osteoporosis (defined as low BMD with T-score < - 1.0 SD and prevalent vertebral fractures) was present in 22%, even in those aged less than 30 years (1). A 40% greater incidence of fractures was found among IBD patients than among the general population (104). Another study showed a significantly increased risk of low energy fractures in female patients with CD (15.7% vs. 1.4% in controls) (105).

Pediatric IBD patients

The prevalence of low BMD also showed great variation in the pediatric IBD group. BMD Z-scores < - 1.0 SD were present in 26-70% of the patients and Z-scores < - 2.0 SD in 5-39% (10, 12, 106). As in most studies of adults, pediatric cross-sectional studies reported a significantly lower BMD in CD than in UC patients (8, 10). Others, however, have been unable to support this finding (107). Several pediatric studies have focused only on CD patients (12, 13, 108). Moreover, it has been demonstrated that in CD patients with growth retardation BMD should be corrected for bone age (12, 13) or bone size (106).

A few longitudinal pediatric studies with varying results have been published (9, 95, 109, 110). Gupta et al. (110) found significantly lower BMD both in CD and UC patients but no statistically significant change in the lumbar spine during follow-ups of between 1.7 and 8.7 years. In the study conducted by Sylvester and co-workers (95), lumbar spine and total body BMD in newly
diagnosed CD patients was low and stable during a two-year period. In UC patients BMD was at the same low level as in CD patients but it increased significantly between one and two years of follow-up. In Boot’s study too, BMD was significantly decreased at baseline (9). No improvement was observed in the entire group of IBD patients at one year follow-up, but after two years a significant improvement in BMD Z-score was seen. A more recent longitudinal study using pQCT technique found significant deficits in trabecular vBMD at the time of diagnosis in CD patients aged between 5 and 18 years (109). At follow-up two years later, trabecular vBMD had improved but was still significantly decreased compared to controls.

The issue of fractures in the pediatric IBD group has been presented only in a few case reports (7, 111, 112) and the study by Persad et al. (113). In the case reports the occurrence of vertebral fractures was described as an unusual presentation of childhood CD (112) or they were found very early in the disease course (111). Surprisingly, Persad and colleagues found in a survey no statistically significant difference in the prevalence of fractures in pediatric IBD patients as compared with their healthy siblings.

Pathogenesis of decreased BMD in IBD

The pathogenesis of decreased BMD in IBD has been regarded as multifactorial. It includes factors such as the inflammation *per se* (23), treatment with corticosteroids (24), low body weight (25), vitamin D deficiency (26) and genetics (27).

The observation that low BMD was already present in patients with newly diagnosed IBD prior to any treatment (112) suggests that the inflammatory process itself may play a role in the
Theoretical Background

pathogenesis of disturbed bone mineralization in this patient group. Animal models support this assumption. In a rat model, colitis was induced and rapid bone loss was observed after only three weeks (114). Interestingly, the bone loss proved to reverse as the colitis healed. In another study, organ cultures of fetal rat parietal bones were incubated with sera from children with CD, UC and controls (115). Bone formation was impaired when the cultures were exposed to sera from patients with CD, but this was not seen when sera from UC patients or controls were used.

Tumor necrosis factor-α

One of the proposed key contributors in both mucosal inflammation and the pathophysiology of bone loss is TNF-α (116). TNF-α is not only necessary for stimulation of osteoclastogenesis together with RANKL but it also inhibits the activation of osteoblasts from their progenitor cells. Furthermore, it stimulates the expression of genes that amplify osteoclastogenesis (IL-6, M-CSF) but also inhibits those that are involved in bone formation (ALP, vitamin D receptor, PTH receptor). TNF-α may also induce resistance to vitamin D, which also affects skeletal metabolism (117). As a clinical application of these findings, treatment with antibodies against TNF-α (infliximab) was found to influence the clinical course of IBD patients but also to improve their BMD (103). In a recent study it was demonstrated that the TNF-α haplotype is strongly associated with BMD in patients with CD (118). Another important finding is that the gene that encodes TNF-α is located in the HLA III region of the HLA complex (119). This region is part of the IBD3 on chromosome 6p21.1-23 whose role in determining susceptibility and phenotype of IBD has been extensively
investigated. Other involved cytokines are IL-1β (120) and IL-6 (27, 121).

**RANKL-OPG system**

As described, the RANKL-OPG system is the major system that balances bone formation and bone resorption (122). It has been shown that the RANKL-OPG system is activated in adult IBD and it relates to the degree of bone loss (123). In a population-based case control study, serum OPG was found to be increased in CD patients (124). The finding from both studies – that OPG is regulated upwards in IBD - may contradict its role in the system but this may be explained as an attempt to maintain normal bone mass. Activated T cells, which are central in IBD, can also trigger this system (51) and thus establish the link between bone metabolism and autoimmune disease. Novel functions of RANK(L) signaling have been described recently (125), and these discoveries shed new light on the crosstalk between bone and the immune system.

**Corticosteroids**

Corticosteroids are important regulators of diverse physiological systems and they have been used in the treatment of several inflammatory diseases. Their deleterious effect on bone and growth has been known for a long time and was described first by Cushing in 1931 (126). Baron and co-workers were able to demonstrate a direct inhibiting effect on the growth plate when they administered dexamethasone directly into the epiphyseal growth plates of rabbits (127). Another interesting observation is that individuals with familial glucocorticoid deficiency are taller than the normal population (128). Recent research has shown that high doses of corticosteroids decrease osteoblastogenesis and
Theoretical Background

increase the apoptosis of osteoblasts and osteocytes. This in turn reduces the amount of newly formed bone (129). Furthermore, corticosteroids also decrease OPG production and increase RANKL, which results in an increase in osteoclasts (130). Other effects are increased calcium loss through the kidneys and the gut, which gives rise to increased osteoclast activity and secondary hyperparathyroidism, and increased somatostatin concentration, which results in decreased release of growth hormone. Further, direct effects on the growth plate, alterations in gonadal function at the level of the pituitary gland and direct effects on gonads have been described (131). Corticosteroids are an important treatment option for active IBD. Attempts have been made to find the optimal dosage in order to minimize side effects in pediatric patients (108).

Vitamin D metabolism

Vitamin D is not only essential for bone mineralization throughout life but is also considered to regulate the immune system, for example in IBD (132). The most important source of vitamin D seems to be endogenous skin production (133). This assumption is supported by the fact that levels vary according to season and significantly lower levels are found during winter time. This is reported to be a problem in populations living in the northern hemisphere (67, 134). At latitudes between 30 and 60° N the production of vitamin D in the skin is considered to be insufficient in winter, and at latitudes over 60° N no vitamin D at all is produced in winter.

Vitamin D deficiency has been described in adult IBD patients, especially in CD (2, 26). In pediatric patients data are limited and conflicting. Whereas two studies have reported vitamin D levels to
be in the normal range (10, 108), another study has found vitamin D deficiency in more than one third of pediatric patients with IBD (135). It has been hypothesized that malabsorption of vitamin D might play a role but vitamin D absorption in CD patients seems not to be compromised (136). However, some IBD patients also suffer from liver diseases such as autoimmune hepatitis and primary sclerosing cholangitis, which are known to compromise vitamin D absorption and metabolism. Moreover, it has been suggested that TNF-α may induce vitamin D resistance in IBD (116). As a key player in inflammation in IBD, TNF-α has been shown to decrease vitamin D receptor count and vitamin D stimulated receptor transactivation in osteoblastic cells (117, 137). When the relationship between vitamin D levels and BMD was examined, a significant correlation was found in some studies (138, 139) but not in others (140, 141).

Parathyroid hormone

PTH is another important regulator of bone metabolism and it is primarily responsible for maintaining a stable serum calcium concentration. This is achieved through various mechanisms, such as activation of osteoclasts, inhibition of phosphate resorption in the kidneys or induction of vitamin D production. Studies have shown varying results for PTH levels in adult IBD patients. Some studies have noted normal levels (138), whereas others have found increased levels of PTH (139). Only limited data exist on the PTH status of children with IBD. Issenman et al. (108) described an increase in PTH after disease treatment. Gokhale et al. (10) observed lower PTH in CD than in UC patients. The relationship between PTH and BMD was not investigated in these studies.
Body composition in pediatric IBD

Body composition is of special interest since it reflects the nutritional status of IBD patients and yields detailed information about the distribution of lean mass, fat mass and BMC. This information can easily be obtained from DXA measurements. However, interpretation of the data is still difficult as there are only a few pediatric reference materials available (142-144).

In IBD, lean mass deficits have been described in both children (145) and adults (146), especially in those with CD. It was also demonstrated that these deficits tended to persist over time (147). These findings may be caused by the inflammation per se (148), treatment with corticosteroids, decreased physical activity and / or malnutrition.

Growth failure in pediatric IBD

Even the first descriptions of the disease identified growth failure as a characteristic of IBD (149, 150) and this is unique to pediatric patients. Features of growth failure include linear growth impairment and delayed bone age. Linear growth impairment has been defined according to reduced height Z-score or height velocity < - 2.0 SD and it has been reported to be present in up to 65% of CD patients (35, 151, 152). Final adult height below target height has also been used to describe growth impairment in IBD patients and it was present in every third to every fifth patient (35, 153).

Normal postnatal growth is regulated by GH, IGF-1, thyroxine and sex steroids, whereas the pubertal growth spurt is primarily induced by estrogen. The pathogenesis of growth failure in IBD is regarded as multifactorial (154). Special note has been made of
the effect of proinflammatory cytokines in IBD that contribute to the disruption of the GH-IGF-1 axis and to GH resistance. The mechanisms behind this are not completely understood. Another important factor is the chronic malnutrition seen in pediatric patients, especially those with CD. Malnutrition plays a role in the suppression of IGF-1 (155). Moreover, patients are often treated with corticosteroids, which impair the linear growth through a functional GH deficiency (156).

The management of growth failure requires a multidisciplinary approach and the optimum usage of the available treatment options including nutritional therapy, immunomodulators (e.g. azathioprine, methotrexate), anti TNF-α therapy and surgery (42). GH therapy is still considered to be experimental but has shown to be effective in individual patients with CD and growth failure (156).

Evaluation and treatment of decreased BMD in IBD patients

Guidelines have been published regarding evaluation and treatment of osteoporosis in adults with IBD (157, 158) but no corresponding recommendations exist for pediatric IBD.

Evaluation of BMD in adult IBD patients

Some investigators have suggested that all individuals with IBD should have a DXA at some point because of the high occurrence of bone loss in this patient group and the burden of osteoporosis (159). The guidelines of the Crohn’s and Colitis Foundation of America (CCFA) support this suggestion (158). It is therefore recommended that all patients should be screened with DXA soon after their diagnosis and that this should be repeated after 12 to 18 months. Special attention should be paid to those with a
previous history of fracture, those older than 65 years, and other risk factors such as use of corticosteroids.

**Treatment with calcium and vitamin D**

A Cochrane review recently stated that supplementation with calcium and vitamin D may reduce the risk of fracture in an elderly population without IBD, although the efficacy of vitamin D as the sole method of fracture prevention was still unclear (160).

There are few studies available of adults with IBD that compare the effect of vitamin D supplementation and calcium alone to that of combined vitamin D, calcium and anti-resorptive agents (161). It seems as though treatment with vitamin D and calcium increases BMD, but that additional treatment with fluoride or bisphosphonates does not bring any additional benefits. Benchimol and colleagues (162) found in the only study of pediatric IBD patients that supplementation with vitamin D and calcium did not accelerate the increase in BMD. To my knowledge, no studies of IBD patients have investigated the role of supplementation in fracture prevention.

**Treatment with bisphosphonates**

Bisphosphonates induce apoptosis in osteoclasts (163) and thereby enhance bone formation. Whereas bisphosphonates are widely used in postmenopausal osteoporosis, only a few studies have investigated the effect of bisphosphonates in adult IBD patients (164, 165). Bisphosphonates were shown to improve BMD in these studies, but no data are available for pediatric IBD patients.
General recommendations

As stated in the CCFA guidelines (158), the use of corticosteroids should be kept to a minimum while other effective therapies should be used optimally. Further, adequate calcium intake should be ensured, regular physical activity advocated and patients should be advised to stop smoking and reduce their alcohol consumption. If there is deficiency, vitamin D should be supplemented.
Aims

- To investigate BMD in a population of Swedish children and adolescents with IBD and to evaluate possible factors affecting BMD.

- To investigate the familial resemblance of BMD in pediatric IBD patients.

- To follow up the BMD in this population after two years and to identify factors that may influence changes in BMD during this period.

- To investigate body composition and linear growth of pediatric IBD patients focusing particularly on differences between CD and UC and assessing possible changes over time.
Patients and Methods

Patients and study design

This thesis was designed as a prospective, longitudinal, population-based study with patients from two pediatric centres in a region of Western Sweden. The two centres are the Queen Silvia Children’s Hospital at Sahlgrenska University Hospital, Gothenburg, and the Department of Pediatrics at Borås Central Hospital. These are the only centres in this region that are responsible for the diagnostic work-up, treatment and follow-up of IBD patients.

During a two-year period between 1 January 2003 and 1 January 2005 we identified 166 eligible patients. Inclusion criteria were age between 6 and 19 years and an existing diagnosis of IBD. We also included all children and adolescents who were diagnosed with IBD during the inclusion period. Of the 166 identified patients, 22 (13.2%) did not participate in the study. There were no differences regarding gender, disease subcategories, disease duration or treatment between the participating and non-participating groups.

The diagnosis of IBD was made on the basis of the Porto Criteria (38). According to these criteria the diagnosis of IBD, including CD, UC and IC, is based on clinical signs and symptoms, endoscopy, histology and radiology.

At baseline, a total of 144 IBD patients (93 males and 51 females, mean age 14.2 years, range 6-19 years) were enrolled: 83 with UC, 45 with CD and 16 with IC. Additionally, we included 266 biological parents (136 mothers and 130 fathers) at baseline. In the group of the included parents, 8 mothers and 8 fathers had a
self-reported previous history of IBD (6%), in each gender group there were 6 with UC and 2 with CD.

At follow-up two years later (mean time for follow-up 24.6 months, range 15-33 months), 126 of the initial 144 pediatric patients (81 males and 45 females, mean age 16.6 years, range 8-22 years) were re-examined: 75 with UC, 37 with CD and 14 with IC. Those 18 patients who did not appear for follow-up did not differ from the participating group in terms of diagnosis, gender or BMD Z-score for the lumbar spine at the first DXA measurement, but they were less likely to be using corticosteroids (44.4% vs. 80.6%, p<0.001).

The patients were included in the study through the year. Venous blood samples were taken at regular visits throughout the day. These visits were scheduled to take place close to the time of the DXA measurements. Samples were obtained from 131 patients at the first DXA measurement and from 118 patients at the second DXA measurement. The blood was centrifuged and the serum stored frozen at -20°C before processing.

**BMD measurements**

To evaluate BMD, the patients underwent DXA of the whole body, lumbar spine and femoral neck at the time of inclusion in the study and two years later at follow-up. The included parents were only examined with DXA at baseline. All patients’ and parents’ measurements during the study period were performed on the same densitometer at Sahlgrenska University Hospital in Gothenburg, applying a Lunar® densitometer (General Electric Medical Systems, Lunar; 726 Heartland Trial, Madison, WI 53717-1915, USA). BMD values in the pediatric group were expressed as Z-scores using pediatric gender-specific and age-matched reference data from Lunar® (166). Pediatric referencing data were
however lacking for BMD of the femoral neck. Adult reference values from Lunar© were used for the adult group. For ethical reasons, we decided to supplement pediatric patients who had BMD Z-scores < -1.0 SD on their first DXA measurement with 1,000 mg calcium and 800 IE vitamin D daily.

**Body composition measurements**

The total body scan obtained by DXA at baseline and follow-up (as described above) provided data not only for BMD but also for body composition as follows: BMC, fat mass and lean mass. Fat mass and lean mass were expressed in kilograms (kg) and percent body weight.

**References**

As stated in *Bone mineral density measurements* we used the gender-specific and aged-matched pediatric reference data provided by Lunar© as controls (166). These reference data were obtained from six studies (167-172) that included Caucasian volunteers from five different countries (Netherlands, Spain, Finland, Australia and USA) between the ages of 5 and 19 who had no condition that was known to affect bone mineral density. The entire reference material consisted of 1,135 female and 924 male subjects with DXA scans of the lumbar spine (L2-L4) and 821 female and 673 male subjects with total body scans.

As references for body composition we used values from healthy adolescents provided by Lantz and colleagues (142) for the ages 15 (mean age 15.06 years, ±0.12 SD; 93 boys, 109 girls), 17 (mean age 17.11 years, ±0.10 SD; 65 boys, 84 girls), and 20.5 years (mean age 20.55 years, ±0.37 SD; 50 boys, 56 girls). These individuals had been randomly selected from the official
population register at the town of Trollhättan which is situated approximately 80 km northeast of Gothenburg. The criterion for inclusion was the absence of chronic diseases. DXA in this group was, as in the present study, also obtained by a Lunar\textsuperscript{©} densitometer. Local references for children below 15 years of age were not available.

A Swedish reference material was used for comparison of age at peak height velocity (PHV) (173). This group had been recruited in 1992 in the city of Gothenburg and it consisted of 1,208 boys and 1,182 girls of around 18 years of age.

Estimation of pubertal stages

The assessment of pubertal stages according to the following classification is the sum of Tanner stage, age at PHV if available (depending on patients’ age), bone age and serum estradiol or testosterone levels. Pubertal stages were classified by a pediatrician (S.S.) under the supervision of an experienced senior pediatric endocrinologist (E.N.) as P1 to P5 (P1 = prepubertal; P2 = pubertal, but more than six months before PHV; P3 = six months before or after PHV; P4 = more than six months after PHV but still with a growth velocity of >1.5 cm/ last year; P5 = adult status with a growth velocity <1.5 cm/ last year).

Two years after completion of the follow-up study growth charts from all 144 patients could be obtained retrospectively. Calculation of age at PHV was possible in 82 patients (58 boys, 24 girls; 43 with UC, 30 with CD, 9 with IC). Age at PHV was calculated according to the Infancy-Childhood-Puberty (ICP) model (174), using software developed by Kindblom and colleagues (175). It was not possible to calculate age at PHV in 62
Patients and Methods

patients; 16 of them were still prepubertal or pubertal and 46 of the charts did not contain a sufficient number of observations.

Bone age was estimated by means of a radiograph of the left wrist using the Tanner-Whitehouse 2 method (176).

Serum testosterone concentrations were determined in duplicate using a modified radioimmunoassay (RIA) (Spectria® testosterone; Orion Diagnostica, Espoo, Finland) (177). Assay sensitivity was 0.03 nmol/L. The intra-assay coefficient of variation (CV) was 11% for concentrations of 0.2 nmol/L and below 7% for concentrations above 0.4 nmol/L. The inter-assay CV was 16% for the concentration of 0.2 nmol/L and below 10% for concentrations above 0.8 nmol/L.

Serum estradiol concentrations were determined in duplicate by an assay which involved a diethyl ether extraction step prior to quantification by a modified commercial RIA (Spectria® Oestradiol RIA; Orion Diagnostica, Espoo, Finland). With an analytical detection limit of 4 pmol/L, this assay is sensitive enough to determine low prepubertal levels in girls (178). The intra-assay CV was 10-17% in the 5-37 pmol/L range, while the inter-assay CV was 19% at 6 pmol/l and below 14% for concentrations of 12 pmol/L and above. The analyses of both estradiol and testosterone were conducted at the laboratory of the Göteborg Pediatric Growth Research Center; the methods have been accredited by SWEDAC (SS-EN ISO 15189, no.1899).

Vitamin D and PTH measurements

Vitamin D status was assessed by serum 25-hydroxyvitamin D (25-OHD) using a competitive RIA (DiaSorin, Stillwater, MN, USA). This assay measures the sum of 25-OHD3 and 25-OHD2.
Patients and Methods

The intra and inter-assay CV were 6% and 13-16%, respectively. The analyses were conducted at the Hormone Laboratory at Aker University Hospital (Oslo, Norway), where the reference range for serum 25-OHD is 37-131 nmol/l for both children and adults. The samples were measured in singlicate. The method is certified by the Norwegian Accreditation Board (NS-EN ISO/IEC 17025). Following earlier studies (179), we defined vitamin D deficiency as a value < 25 nmol/l.

The serum levels of intact PTH (iPTH) were measured using a non-competitive immunoluminometric assay (Immulite 2500, Siemens Healthcare Diagnostics, Los Angeles, CA, USA). The intra and inter-assay CVs were 3-5% and 7-11%, respectively. These analyses were also conducted at the Hormone Laboratory at Aker University Hospital (Oslo, Norway), where the reference range for iPTH is given as 1.5-7.0 pmol/l for those between the ages of 18 and 65 years. Pediatric reference values were provided by Coffi et al. (180). The samples were measured in singlicate. The method is also accredited by the Norwegian Accreditation Board (NS-EN ISO/IEC 17025).

Serum total calcium, phosphate, albumine and creatinine were measured by certified methods (SWEDAC, SS-EN ISO 15189, no.1240) available at Sahlgrenska University Hospital Laboratory in Gothenburg (Sweden).

Additional data

In addition to the above-mentioned basic clinical data, such as age, gender, disease subcategory (UC, CD or IC), disease duration and treatment (corticosteroids, azathioprine), we collected data on body weight, height, BMI and Tanner stage. With regard to treatment with corticosteroids or azathioprine, we
Ethical considerations

recorded whether the patient had ever taken these drugs without regard to daily or cumulative doses.

Ethical considerations

Assent was obtained from the younger children and informed consent from adolescents as well as written permission from the parents. The study was approved by the Ethical Committee of the University of Gothenburg (Sweden).
Results

Since this thesis will be available electronically, the results are presented only briefly here so as not to hinder later publication of original papers. The results are presented in greater detail in the attached papers and manuscripts.

Study I and III

In this population-based study, the lowest BMD values were found in the lumbar spine. At baseline the entire IBD group showed significantly lower BMD Z-scores for the lumbar spine (L2-L4) than healthy references (-0.8 SD, range -5.9 to 3.7 SD, p<0.001). These values remained unchanged at follow-up. Significantly decreased BMD Z-scores were seen in patients with CD as well as UC both at baseline and follow-up. No significant differences in BMD were found between CD and UC patients. Furthermore, BMD mean Z-score for the lumbar spine was significantly decreased in both males (-1.0 SD, ±1.6 SD, p<0.001) and females (-0.4 SD, ±1.5 SD, p<0.05) at baseline. This finding persisted unchanged at follow-up.

Subanalyses for the different age groups were made. At baseline, the lowest BMD values were seen in the age group 17-19 years in males (mean Z-score lumbar spine -1.47 SD, ±1.9 SD) as well as in females (mean Z-score lumbar spine -2.06 SD, ±1.3 SD). At follow-up, both males and females had improved their BMD Z-scores significantly. In a multiple regression model with the BMD of the lumbar spine as the dependent variable, the factors possibly associated with lower BMD were male gender, low BMI and treatment with azathioprine. Investigation of the factors influencing the change in the BMD of the lumbar spine between
the two measurements, patients with disease duration < 12 months at baseline showed a significantly lower mean change of BMD of the lumbar spine.

**Study II**

The BMD of the IBD children was clearly related to the BMD of their parents. The strongest correlation between the BMD of the children and the mid-parent value was found in the femoral neck with r=0.55 (p<0.001, 95% CI 0.41-0.66). The group of children with IBD had an Odds ratio of 5.96 for decreased BMD (lumbar spine Z-score < -1.0 SD) given that decreased BMD was diagnosed in both parents.

**Study IV**

Significantly lower lean mass was found in the group of males in late adolescence (19-22 years) than in healthy references. This finding was accompanied by significantly lower body weight and preserved fat mass. In females, a decrease in lean mass and body weight could also be observed in the same age group, but these differences did not attain statistical significance. In a linear regression model for all IBD patients in late adolescence with lean mass at follow-up as the dependent variable, CD patients showed significantly lower lean mass as well as lower body weight than UC patients. In males as well as in females mean age at PHV was significantly delayed, compared to healthy references. Linear growth impairment was not common in the group of our patients.
Discussion

Low BMD is recognized as a potential complication in pediatric IBD. However, the prevalence reported in pediatric studies is highly variable. The pathogenesis of low BMD in pediatric IBD remains unclear but the inflammation per se seems to be a strong contributing factor. The discussion below focuses on the thesis’ main findings and on their clinical implications.

Prevalence of low BMD in pediatric IBD

In this longitudinal population-based study, low BMD was found to be frequently present in patients with pediatric IBD. To my knowledge, this is the first population-based pediatric study in this field. BMD Z-scores < - 1.0 SD occurred in almost 50% of our patients and BMD Z-scores ≤ - 2.0 SD in a fourth (paper I). These findings are in the same range as previously reported figures, where BMD Z-scores < - 1.0 SD were present in 26-70% of the patients and Z-scores < - 2.0 SD in 5-39% (10, 12, 106). These variations may in part be explained by different methods of patient selection, investigation of different disease subgroups, ethnic variations or the references used. Another possible explanation is underestimation of BMD by DXA in patients with impaired growth, like some of our pediatric IBD patients. It is possible to compensate for these inaccuracies by correcting BMD values for bone age. Herzog and co-workers (13) showed that the figure for the patients with abnormal BMD Z-score < - 2.0 SD fell from 44% to 26-30% when this adjustment was performed. In our group of patients, bone age was delayed by at least one year in 19.1% (paper I). Those with delayed bone age had a lower BMD mean Z-score of the lumbar spine than the patients with normal bone age (-1.4 SD vs. -0.6 SD). When bone age was used instead of
Discussion

chronological age as the reference age, BMD mean Z-scores for the lumbar spine increased from -1.4 SD to -0.8 SD in those with delayed bone age.

Low BMD has also been reported in other chronic diseases that begin early in life, such as cystic fibrosis (17), insulin-dependent diabetes mellitus (16) or juvenile rheumatoid arthritis (181). It is difficult to compare the figures reported from these studies with our results, but the prevalence of BMD ≤ - 2.0 SD seems to be higher in our study. This may partly be explained by different immune mechanisms involved in these diseases that in turn have different effects on bone metabolism.

BMD and disease subcategory

In the present study both CD and UC patients had low BMD values (-1.1 SD respectively -0.8 SD) at baseline. CD patients tended overall to have lower BMD. However, no statistically significant difference in BMD between the disease subcategories could be established (paper I). In accordance with our results, a study of adults with IBD conducted by von Tirpitz et al. (4) found low BMD in both CD and UC patients but no significant difference between the groups. By contrast, earlier studies of children and adolescents with IBD have reported lower BMD in CD than in UC patients (9, 10). These differences in BMD between CD and UC patients may be explained by different immune mechanisms. CD and UC patients show miscellaneous cytokine patterns in the mucosa, and this indicates that different immune responses are involved in each of these two diseases (182). One of the most important cytokines in CD, TNF-α, has been shown to be involved in bone metabolism as well (116).
**BMD and disease activity**

Interestingly, treatment with azathioprine turned out to be a factor associated with lower BMD in the present study (paper I). In Sweden, azathioprine is given quite early in the disease course as a supplement in a “step-up-strategy” for relapsing or steroid-dependent disease. Azathioprine is not known to have any effect on bone turnover. Since it is used in children with more active disease we consider it to be a marker of disease course severity. This assumption is in agreement with a previous report (24) that demonstrated that treatment with 6-mercaptopurine (6-MP), a major metabolite of azathioprine, was a significant risk factor for low BMD. The authors suggested that the use of 6-MP was an indicator of disease severity since it is used in refractory CD.

A number of markers of disease activity have been used, such as the Pediatric Crohn Disease Activity Index (PCDAI), hospital admissions, length of stay at the hospital, hypoalbuminemia or total parental nutrition (23, 24). We assessed our CD patients with PCDAI at the time of inclusion in the study (data not shown). Almost all our patients scored very low, which indicates that they were in remission. PCDAI assesses the patients’ condition over the last week and not over a longer period of time. However, since BMD changes occur over time it did not seem useful to apply PCDAI in this context. Nor did the other proposed markers seem applicable since patients nowadays are rarely admitted for hospital care.

**BMD changes over time**

We found a decreased but stable BMD Z-score of the lumbar spine in both the CD and UC patients over time (paper III). Our data show that our pediatric patients increased their absolute
BMD since most of them were still growing but they did so along a lower Z-score line. Longitudinal pediatric data on this topic are limited and conflicting (9, 95, 110). While one study supports our findings (110), others have observed a significant improvement in BMD Z-score in the lumbar spine in UC but not in CD patients (95). A further study noted improvement in BMD Z-score for the entire group of IBD patients (9). A more recent longitudinal study, using pQCT technique, found significant deficits in trabecular vBMD at diagnosis in incident pediatric CD patients (109). These deficits persisted after two years of follow-up with only slight improvement. The diverging results of the above-mentioned studies may result from different patient selection procedures or sample sizes.

An explanation for the stability of BMD over time may be the fact that the RANKL-OPG system is able to regulate itself upwards. This was shown in adult IBD patients (123). RANKL is activated by the overexpression of proinflammatory cytokines, particularly TNF-α, and initiates bone resorption. As a compensatory mechanism to improve bone formation, OPG is then regulated upwards such that no further bone loss ensues.

Potential for recovery of BMD from late adolescence into early adulthood

Our longitudinal data indicate that increase in BMD may continue even beyond adolescence in patients with IBD (paper III). This means that recovery of BMD may continue into early adulthood. We analyzed separately the group of patients who had completed their pubertal development and were growing less than 1.5 cm per year at the time of the first DXA measurement. In this group we could show that the patients continued gaining BMD in the lumbar
spine and this resulted in improved BMD Z-scores for the lumbar spine with around 0.5 SD at the end of the study period. This was particularly clear in males; the small size of the female sample made it difficult to draw any firm conclusions. In healthy adolescents, increase in bone mass is almost complete in the lumbar spine in females by the age of 16 (63) and in males by 18-20 years of age (64). This is also seen in large reference groups (143, 166). The results of the study of Bernstein et al. (183) indicate more indirectly the potential of recovery in BMD of IBD patients. He compared a group of 12 women with a prepubertal onset of the disease with a group of 58 women with postpubertal onset but before 20 years of age. It was concluded, that regardless of whether IBD presented before or after puberty, it had little impact on the ultimate peak bone mass achieved in adulthood.

This potential for recovery may be explained by delayed and / or prolonged puberty. As we found in our IBD patients, age at PHV is significantly delayed in both males and females (paper IV). Further, bone age, another parameter of pubertal development, was delayed in our prepubertal patients. Growth-inhibiting conditions such as IBD slow chondrocyte proliferation and thus growth plate senescence. If the growth-inhibiting condition then stops, the growth plate is less senescent and growth will occur more rapidly than normal; this is known as catch-up growth and was demonstrated by Gafni and colleagues (80) in a rabbit model after the administration of corticosteroids. In our study, the catch-up in linear growth seemed to be followed by catch-up in bone mineralization.
BMD and corticosteroid therapy

We found no significant correlation between corticosteroid treatment and reduced BMD (paper I) or between corticosteroid treatment and lower change in BMD (paper III). This might partly be due to the fact that long-term treatment with corticosteroids is not frequently used in the modern therapy of pediatric IBD. Usually a course lasts up to 12 weeks. Further, steroid-dependent or frequently relapsing disease is treated early with immunomodulators, such as azathioprine or 6-MP. However, there seems to be no doubt that corticosteroids have a deleterious effect on bone (126), but it remains unclear what influence timing, duration and/or dosage may have in children (131). There are no data available concerning the risk of fractures in pediatric patients with IBD who have been treated with corticosteroids. Children with other conditions, such as asthma, who have received frequent, short-term courses of oral corticosteroids have shown an increased risk of fracture with an OR of 1.32 (184). Interestingly, the risk of fracture became comparable to that of controls once the treatment had been discontinued.

Familial resemblance of BMD

We were able to demonstrate that the BMD of pediatric IBD patients is significantly related to the BMD of their parents (paper II). The strongest correlations were found between the child’s values and mid-parent BMD values. It is noteworthy that all correlations with the mid-parent BMD values were highly significant and this indicates that both parents have a strong influence on the BMD of their children. Our results are in accordance with previously reported figures from healthy families and they underscore the fact that familial resemblance of BMD is
Discussion

present in pediatric IBD patients despite a chronic inflammatory condition.

Familial resemblance refers to the factors that influence the variance of BMD in a population. These consist of genetic and environmental factors. Early family studies of mother-daughter pairs found correlation coefficients of BMD between 0.22 and 0.39, depending on skeletal site (72, 185). In the first daughter-parent study (70), the highest correlations in bone mass were seen between the mid-parent values ([mother’s BMD + father’s BMD] / 2) and the daughter’s value (r between 0.60 and 0.72, depending on skeletal site). From this study it was concluded that bone mass in young women is influenced by genetic information not only from the mother but from both parents. Another study of parents and their offspring showed that mid-parent BMD Z-scores were positively correlated with those of daughters as well as sons and that the correlations with the mid-parent value were higher than with that of mother or father alone (r between 0.34 and 0.58) (186). A Swedish study of parents and their adolescent sons estimated that genetic factors account for about 34-54% of the variation in the sons’ BMD (74). The corresponding figure for our study would be around 30%.

We were also able to show that the Odds ratio of having reduced BMD increased markedly if both parents had reduced BMD. This finding is in accordance with the findings of another parent-offspring study of healthy individuals, which had approximately the same sample size as ours (75). This French study found that the relative risk of having low BMD was 4.3 if a child had one parent with low BMD, but it increased to 8.6 when both parents had low BMD.
As far as we know, ours is the first study detailing the familial resemblance of BMD in children and adolescents with IBD. One limitation is that we were unable to control for potentially influential environmental factors. In the statistical model we adjusted BMD for age, sex, body weight, body height and parental IBD. However, we found no correlation between the BMD of the mothers and that of the fathers. This may rule out any analytical bias being responsible for inter-family correlations.

**Clinical significance of low BMD**

Since we did not elicit information about fractures, this thesis cannot provide any answers to the question of prevalence of fractures in pediatric IBD patients. Pediatric data on this topic are limited (112, 113). Further studies are required in order to assess whether or not the risk of fracture is increased.

It has also been hypothesized that peak bone mass may be reduced in IBD patients with pediatric onset of the disease. However, our study shows that genetic factors also play an important role, even in the presence of a chronic inflammatory disease (paper II) and that catch-up in bone mineralization may also continue into early adulthood (paper III). These findings suggest that pediatric IBD patients may have a good chance of achieving their “normal”, genetically determined bone mass.

**Vitamin D and parathyroid hormone**

The prevalence of vitamin D deficiency varies according to populations. In adults and children with IBD both normal (11, 100) and reduced (10, 26) vitamin D levels have been reported. We employed the broadly accepted definition of vitamin D deficiency as < 25 nmol/l and found that only 2.3% of our patients at baseline
and 4.1% at follow-up were vitamin D deficient (paper III). There is growing evidence that sequelae of vitamin D deficiency may manifest at higher vitamin levels of 50 nmol/l or even higher around 80 nmol/l (187). If these new definitions were employed, a considerably larger proportion of our patients would qualify as vitamin D deficient: at baseline 22.1% respectively 62.6% and at follow-up 25.4% respectively 68.6%. However, both the lack of consensus about the definition and the lack of reliable methods for measuring vitamin D levels make this problematic. At present, the variability between different assay techniques may differ considerably.

In likeness to previous reports (67), we found a significant seasonal variation with lower levels of vitamin D in winter. This may be explained by the fact that the area of Gothenburg, in which our study was conducted, is situated at a latitude of 57.7° N. The production of vitamin D in the skin between latitudes 30°-60° N is generally considered to be inadequate in winter. Traditionally, Swedish food is rich in fatty fish and dairy products and these provide external sources of vitamin D. We did not take into account the fact that the food habits of our patients may differ from the traditional diet. Nor did we calculate their daily calcium and vitamin D intake and this may have yielded additional, relevant information.

Little is known about the effect of vitamin D and calcium supplementation in pediatric IBD patients. In an open-label, prospective study, Benchimol et al. (162) followed pediatric patients with IBD who showed a BMD Z-score of the lumbar spine < - 1.0 SD over a 12 months period. Patients were supplemented with 50,000 IE vitamin D monthly and 1,000 mg calcium daily or 1,000 mg calcium daily alone. Neither of the intervention groups
showed accelerated increase in lumbar spine BMD when compared to a control group. In our study, we decided to supplement patients with BMD Z-score < -1.0 SD at the first DXA measurement with 800 IE vitamin D and 1,000 mg calcium daily. This had a positive effect on the patients’ vitamin D levels but not on BMD Z-scores for the lumbar spine. I can only speculate at this stage, but a limited function of vitamin D in IBD patients has been proposed (116). It has been shown that TNF-α, which is an important mediator of the inflammation in IBD, not only reduces the number of vitamin D receptors but also inhibits vitamin D stimulated receptor transactivation in osteoblastic cells (117, 137).

Interestingly, we found elevated levels of iPTH in the patients below 16 years of age despite apparent normal vitamin D levels. Abrams and colleagues have reported increased rates of iPTH in healthy adolescents aged 10 to 14 (188) and suggested that in adolescents, especially in the presence of vitamin D insufficiency, PTH secretion increases to adapt to higher rates of bone formation associated with growth. Thus, PTH may be elevated in puberty without indicating insufficient supply of vitamin D or calcium. An elevated PTH concentration may be regarded as a physiological response to the increased calcium and phosphorus demands that accompany the increased growth rate and calcium accretion during puberty. Data on PTH in patients with pediatric IBD are scarce (10, 108). We also observed that the vitamin D and iPTH levels were inversely related in our patient population. This has been described previously for healthy adolescents (188) and adult medical inpatients (189) but not, to my knowledge for pediatric patients with IBD. Vitamin D is required for calcium homeostasis, but in the case of hypovitaminosis D, secondary hyperparathyroidism may develop. In sum, different mechanisms may explain the occurrence of elevated PTH levels in our patients.
Body composition

Our study has shown that in pediatric IBD patients BMD, lean mass and body fat were strongly correlated despite the presence of chronic disease, which could in itself influence body composition (paper I). Lean mass has been reported to be a strong predictor of BMD in healthy children (190). Lean mass consists mainly of muscles. Muscle mass is among other factors dependent on the level of physical activity. The positive effect of physical activity on BMD is well documented in healthy children (191). Data are lacking for children with IBD, but it may be assumed that physical activity has a positive effect on their BMD as well. We did not record the physical activity levels of our patients with an accelerometer, but we obtained data from a questionnaire at the time of their entry into the study (data not shown). Notably, many of our patients were participating in physical education at school and around the half of them were involved in other sports during their leisure time.

Earlier studies in pediatric IBD showed lean mass deficits (145, 192) that persisted over time (147). We found these lean mass deficits only in late adolescence, especially in males with CD (paper IV).

Clinical implications

On the basis of the findings from this study and the evidence from contemporary literature, I would make the following recommendations for the management of BMD in pediatric patients with IBD.

- Since low BMD is prevalent in pediatric IBD, this patient group should be evaluated by DXA. According to the
recommendations for adults, *DXA should be obtained at some point, if possible directly after diagnosing the disease.*

- **Appropriate pediatric references** should be used to obtain BMD Z-scores.

- **BMD Z-scores < -1.0 SD at any skeletal site should be followed up** with new DXA after 1-2 years. At the same time bone age should be obtained and DXA should be corrected for bone age or height when growth failure occurs.

- In patients with low BMD vitamin D status should be checked and deficiency corrected if present.

- **Adequate calcium intake** should be ensured. Patient and parents should meet a nutritionist for further advice.

- **Physical activity**, if possible outdoor.

- **IBD treatment should be optimized** to reduce inflammation. The potential for recovery of BMD gives the pediatric gastroenterologist a greater therapeutic window of opportunity for influencing bone mineralization in the individual patient by optimizing IBD therapy.

- **In patients who show low BMD at repeated measurements** and when no other factors explain these values (high active disease, longstanding use of corticosteroids, vitamin D deficiency), *DXA measurement of both biological parents should be considered* and data about fractures in the family obtained. Since it has been shown that the BMD of both parents influences the BMD of their child, it may be helpful to use parental DXA measurements to interpret a child’s DXA data, also in the case of IBD.
Main conclusions

- Low BMD is highly prevalent and it persists over time in this unselected population of pediatric IBD patients.

- There seems to be a potential for recovery of BMD from late adolescence into early adulthood.

- Family resemblance of BMD is, as in healthy individuals, noted in pediatric IBD patients. This underlines the importance of genetic factors in BMD despite the presence of a chronic inflammatory disease.

- Pediatric patients with IBD should be evaluated with DXA at some point during the course of their disease.
Acknowledgements

While working on this thesis I was in touch with many people who provided inspiration and who contributed in different ways to this work. I would like to extend thanks to all of you and especially to:

All the patients with IBD and their parents who participated in these studies. Special thanks are also due to my very first IBD patient and his family who prompted my interest in bone mineral density.

I also thank my main tutor Robert Saalman, for his unfailing encouragement and optimism over the past years.

I am grateful also to my co-tutor Dan Mellström, who taught me so much about DXA and bone mineral density.

My second co-tutor Ensio Norjavaara gave me new insights into pediatric growth and puberty.

Valter Sundh and Staffan Nilsson provided invaluable statistical advice and help.

All of the staff at the various departments at The Queen Silvia Children’s Hospital Gothenburg (avdelning 334) and the Department of Pediatrics at Central Hospital Borås (barnmottagningen) are due thanks for their help and support. Thanks to all of you and especially to Marie Krantz, Carola Kullberg-Lindh, Audur Gudjonsdottir, Karin Hallberg and Helene Lindfred for help with inclusion and follow-up of the patients.

Senada Catic performed all DXA measurements.
Acknowledgements

Michaela Shapev and Alexandra Kent have provided language advice.

My husband Martin has provided unending patience, support and love throughout these years. Without your help and belief in me, Martin, I would never have been able to finish this thesis.
References


22. Griffiths AM. IBD in Childhood. Inflammatory Bowel Disease Monitor. 2002;3:122-130


32. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989;299:1259-1260


43. Parfitt AM. The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis. Metab Bone Dis Relat Res. 1982;4:1-6

44. Lee SK, Lorenzo JA. Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. Endocrinology. 1999;140:3552-3561


52. Teitelbaum SL. Bone resorption by osteoclasts. Science. 2000;289:1504-1508


60. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy: Osteoporosis prevention, diagnosis, and therapy. JAMA. 2001:785-795


64. Lorentzon M, Mellström D, Ohlsson C. Age of Attainment of Peak Bone Mass Is Site Specific in Swedish Men - The GOOD Study. J Bone Miner Res. 2005;20:1223-1227


77. Ralston SH. Genetic control of susceptibility to osteoporosis. J Clin Endocrinol Metab. 2002;87:2460-2466


79. Gafni RI, Baron J. Childhood Bone Mass Acquisition and Childhood Peak Bone Mass May Not Be Important


82. Valdimarsson O, Alborg HG, Duppe H, et al. Reduced training is associated with increased loss of BMD. J Bone Miner Res. 2005;20:906-912


References


138. Silvennoinen J. Relationships between vitamin D, parathyroid hormone and bone mineral density in...


148. Li YP, Reid MB. NF-kappaB mediates the protein loss induced by TNF-alpha in differentiated skeletal muscle myotubes. Am J Physiol. 2000;279:R1165-1170

149. Crohn BB, Ginzburg L, Oppenheimer GD. Regional ileitis. JAMA. 1932;99:1323-1329


158. Lichtenstein GR, Sands BE, Pazianas M. Prevention and Treatment of Osteoporosis in Inflammatory Bowel Disease. Inflamm Bowel Dis. 2006;12:797-813

159. Papaioannou A, Ferko NC, Adachi JD. All patients with inflammatory bowel disease should have bone density assessment: pro. Inflamm Bowel Dis. 2001;7:158-162; discussion 168-159


166. Wacker W BH, GE Lunar Corporation, Madison, WI USA. Pediatric Reference Data for Male and Female Total Body and Spine BMD and BMC. International Society of Clinical Densitometry. Dallas, TX, USA; 2001


177. Ankarberg-Lindgren C, Norjavaara E. Changes of diurnal rhythm and levels of total and free testosterone secretion from pre to late puberty in boys: testis size of 3 ml is a transition stage to puberty. European journal of endocrinology / European Federation of Endocrine Societies. 2004;151:747-757


191. McKay H, Smith E. Winning the battle against childhood physical inactivity: the key to bone strength? J Bone Miner Res. 2008;23:980-985

Appendix: Paper I – IV