# Bone mineral density and body composition of children and adolescents in health and disease

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# Bone mineral density and body composition of children and adolescents in health and disease

## Botdichtheid en lichaamssamenstelling bij gezonde en zieke kinderen en adolescenten

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# Chapter 1

# General introduction

## Introduction

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk.[1] Osteoporosis is a major public health problem involving postmenopausal women and aging individuals.[2] The lifetime risk of osteoporotic fractures of the vertebral bodies (symptomatic), hip, and distal radius is about 40 % for white women and 13 % for white men.[2] At present, the best possibility to assess the fracture risk of an individual is the measurement of bone mass (g) or bone mineral density (BMD, g/cm$^2$). Studies in postmenopausal women showed that for each standard deviation decrease in BMD there was a 2-3 fold increase in fracture risk.[3,4] Bone mass later in life is determined by the peak bone mass acquired during adolescence and the subsequent rate of bone loss.[5-8] Low peak bone mass results in a higher risk of osteoporosis. A high peak bone mass provides a larger reserve later in life.[9]

BMD increases during childhood until the peak bone mass is achieved, around the age of 18 to 20 years.[10,11] Thereafter, bone mass stabilizes and then decreases progressively in both sexes after 35 to 40 years of age with a steeper decline in women after the menopause[5,12] (Figure 1, adapted from reference 12).

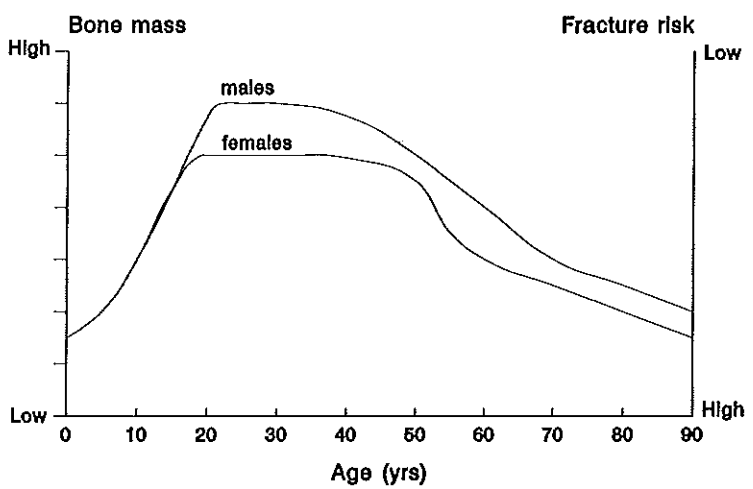


**Figure 1**
*Schematic lifetime presentation of bone mass and fracture risk.*

Children with low BMD have a higher risk of fractures. Besides, they may have a higher risk of osteoporosis in adult life. In children, osteoporosis and low BMD are mainly observed in association with diseases or treatments. Disorders associated with osteoporosis in childhood are listed in Table 1 (adapted from reference 13,14). Preventive measures for osteoporosis later in life is focussed on factors that may increase peak BMD. Therefore, knowledge of determinants of BMD during childhood and adolescence in physiological and pathological conditions is essential with the goal of optimizing peak bone density. Identifying patients at risk of low BMD is important. Optimizing bone mass accretion during childhood and the attainment of peak bone mass reduces the risk of osteoporosis.

**Table 1**
*Classification of osteoporotic conditions of childhood*

| | |
|---|---|
| 1. Endocrine : | hypogonadism |
| | glucocorticoid excess |
| | hyperparathyroidism |
| | hypopituitarism |
| 2. Marrow replacement and expansion | leukemia |
| | anemias (sickle cell, thalassemia) |
| 3. Drugs : | corticosteroids |
| | anticonvulsants |
| 4. Immunologic and inflammatory : | rheumatoid arthritis |
| | inflammatory bowel disease |
| 5. Immobilization : | traumatic paraplegia |
| | cerebral palsy |
| 6. Deficiency states : | vitamin D |
| | calcium |
| | malnutrition |
| 7. Inborn errors of metabolism : | osteogenesis imperfecta |
| | homocystinuria |
| 8. Unknown : | idiopathic juvenile osteoporosis |

## Technique to measure bone mineral density

At present, dual energy x-ray absorptiometry (DXA) is the method of choice to measure BMD. DXA has low radiation exposure, great precision, and accuracy and is

suitable for children.[15] For children below 30 kg in weight special pediatric software has been developed. DXA BMD measurements (Lunar DPXL/PED, Wisconsin, USA) were used in the studies described in this thesis.

Two types of bone can be distinguished in the skeleton : cortical bone, the compact bone of the appendicular skeleton, and trabecular bone, the primary component of vertebral bodies of the axial skeleton and flat bones of the skull and pelvis. Eighty % to 90 % of the volume of compact bone is calcified, whereas 15 % to 25 % of the trabecular bone is calcified; the remainder is occupied by bone marrow, blood vessels and connective tissue.[16] Cortical bone fulfills mainly a mechanical and protective function and trabecular bone a metabolic function.[16] In the studies of this thesis BMD is measured of the total body, 80 % cortical bone, and of lumbar spine, 50 to 70 % trabecular bone.[17]

BMD ($g/cm^2$) measured by DXA is an area density derived from the bone mineral content (g) divided by the projected bone image (area, $cm^2$) of the region. The correction for area removes some, but not all, of the dependency on bone size. Given a fixed volumetric density, large vertebrae have greater BMD values than small vertebrae.[18] To correct completely for bone size, we calculated volumetric density for lumbar spine in some studies. Ancillary DXA-derived data were used to calculate the lumbar spine volumetric bone mineral apparent density (BMAD). The lumbar body was assumed to have a cylindrical shape and BMAD was calculated as follows : Volume = $\pi r^2 h = \pi$ (width/2)$^2$) (area / width).[19] Thus BMAD = BMC / Volume = BMD [ 4 / ($\pi$ width)].[19] The validity of this model was tested by others using magnetic resonance imaging measurements of vertebral dimensions.[19]

## Body composition

Nutritional status is an important indicator of health in children and affects BMD. For many purposes anthropometric measurements as weight for age, weight for height and body mass index provide satisfactory information about the nutritional status of children. However, diseases or treatment may influence body composition which is not reflected in anthropometry. Changes in body fat and lean body mass occur in many disorders. Diseases or drugs which affect bone metabolism may also influence body composition, like growth hormone deficiency or treatment with corticosteroids.

In the studies described in this thesis body composition was assessed by DXA, which has been shown to be a precise and accurate method for assessing body composition.[15,20,21] DXA provides a three compartment model : fat mass, lean tissue mass and bone mineral content.

In two studies, additional bio-electrical impedance was performed, a method which is cheap and easy to perform. It uses a two compartment model : fat and fat free mass. The resistance and reactance are measured by applying a pair of electrodes to one arm and one leg of a subject and using a conduction current of 50 kHz and 800 μA (model 101, RJL systems, Detroit, USA). The measurement is based on the fact that fat free mass contains electrolytes and acts as a conductor while body fat is relatively ion-free and acts as an insulator.

## Bone metabolism

Bone is an active tissue constantly being remodeled in adults as well as in children. Old bone is replaced by new bone. The remodelling process takes place at discrete sites (bone remodelling units). After resorption of a mineralized surface by osteoclasts, osteoblasts are recruited and secrete new bone matrix and gradually fill in the resorption cavity.[22] Bone remodelling is higher in trabecular than in cortical bone. Both systemic and local factors influence bone turnover. In the steady state after growth has ceased, the coupling of bone formation and resorption maintains bone mass.[22] Any imbalance may lead to a change of bone mass. In periods of bone loss, rates of resorption exceed formation. During childhood and adolescence growth involves accumulation of bone called bone modelling. Bone modelling is achieved both by appositional growth along periostal surfaces and by the calcification of cartilage in the growth plate.[23] Chondrocytes regulate enchondral bone formation during linear growth. The remodeling of existing mineralized tissue and the modeling of new bone are each ongoing processes in growing children. Both involve bone formation and bone resorption. The biochemical markers of bone turnover are not specific for either the process of bone modeling or skeletal remodeling.[23] In children, biochemical bone markers correlate with growth velocity.[22] The markers are high in periods of increased growth like the first year of life and during the pubertal growth spurt.
Biochemical markers of bone turnover can be measured in blood and urine samples. Assessment of these markers may provide insight in the pathogenesis of osteopenia. The following bone formation and resorption markers were evaluated in the studies described in this thesis :
Markers of bone formation :
- serum alkaline phosphatase. Total alkaline phosphatase in serum includes several isoforms. Alkaline phosphatase is an enzyme not only produced by osteoblasts but also by other tissues, including liver, intestines, and kidney.[24]
- serum osteocalcin. Osteocalcin is a small protein synthesized by osteoblasts, odonto-

blasts and chondrocytes. While osteocalcin is primarily deposited in the extracellular matrix of bone, a small amount enters the circulation.[22] Osteocalcin has a circadian rhythm with higher nocturnal values in comparison with diurnal values.[25]

- serum carboxyterminal of type I procollagen (PICP). Collagen I represents more than 90 % of the organic bone matrix. It is synthesized by osteoblasts as procollagen with amino- and carboxyterminal extension peptides. These extension peptides are cleaved from the molecules to newly formed collagen.[20] Like osteocalcin, PICP shows a circadian rhythm.[25] PICP is cleared by the liver.

Markers of bone resorption :

- urinary hydroxyprolin. Hydroxyprolin is an aminoacid found in collagenous proteins.[24] Only about 10 % of hydroxyproline-containing products from collagen breakdown are excreted in the urine, the majority is reabsorbed by the renal tubules and broken down in the liver.[24] Another disadvantage is that several other sources of hydroxyproline in addition to bone resorption contribute to urinary hydroxyproline, like diet (gelatin) and breakdown of soft connective tissue.[26] Dietary influences can be circumvented by measuring hydroxyproline/creatinine ratio in the first morning void of urine after an overnight fast.[26]
- urinary calcium. The total daily calcium excretion is dependent on calcium intake. Like hydroxyproline, dietary influence can be minimized by measuring calcium/creatinine ratio in the first morning urine.
- serum cross-linked telopeptide of type I collagen (ICTP). ICTP is released during the resorption of bone collagen. ICTP shows a circadian rhythm, like osteocalcin and PICP.[26]

## Scope of the thesis

The overall aim of the studies reported in this thesis was to identify determinants of BMD during childhood and adolescence in healthy subjects and in patients with diseases or treated with drugs affecting bone mineralization. Determinants of body composition were evaluated in healthy children and adolescents. BMD, biochemical markers of bone turnover and body composition were evaluated in several patient groups.

Knowledge of normal physiological variation is necessary to identify pathological changes. Therefore, we conducted a study in healthy children and adolescents in order to acquire reference values and to evaluate determinants of BMD and body composition in physiological conditions (*Chapter 2*).

Puberty is an important period for bone mass acquisition. In children with central

precocious puberty pubertal development starts prematurely and can be inhibited by the administration of gonadotrophin-releasing hormone agonist. *Chapter 3* describes BMD, bone metabolism and body composition of these children before and during treatment with gonadotrophin-releasing hormone agonist.

In children with inflammatory bowel disease BMD may be negatively influenced by treatment with corticosteroids, malnutrition, or the disease itself. *Chapter 4* describes a study in children with inflammatory bowel disease.

We evaluated BMD and bone metabolism after long-term treatment with inhaled corticosteroids in asthmatic children (*chapter 5*).

*Chapter 6* presents a study in children with acute lymphoblastic leukemia. BMD in these children may be affected by the disease or by the treatment of corticosteroids and chemotherapy.

In *chapter 7* studies in patients with renal diseases are described. Treatment with corticosteroids after transplantation or renal osteodystrophy before transplantation may influence bone metabolism. In *chapter 7.1* BMD of young adult patients who received a renal transplantation in childhood was assessed. In *chapter 7.2* BMD and bone metabolism were studied in children with chronic renal insufficiency. Children with chronic renal insufficiency and growth retardation were treated with growth hormone and the effect on BMD, bone turnover and body composition was studied.

In children with growth hormone deficiency BMD, body composition, bone metabolism and lipid metabolism were studied before and during treatment with growth hormone (*chapter 8*).

*Chapter 9* discusses the presented data and suggestions are made for future research.

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# Chapter 2

# Determinants of bone mineral density and body composition in healthy children and adolescents

# Chapter 2.1

# Bone mineral density in children and adolescents: relation to puberty, calcium intake and physical activity

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## Abstract

The association of height, weight, pubertal stage, calcium intake and physical activity with bone mineral density (BMD) was evaluated in 500 children and adolescents (205 boys and 295 girls), aged 4 to 20 years. The BMD (grams per $cm^2$) of lumbar spine and total body was measured with dual energy x-ray absorptiometry. Lumbar spine volumetric BMD was calculated to correct for bone size. BMD and volumetric BMD increased with age. During puberty the age-dependent increment was higher. After adjustment for age, the Tanner stage was significantly associated with all three BMD variables in girls and with spinal BMD in boys. In boys positive correlations were found between BMD and both calcium intake and physical activity, after adjustment for age. Stepwise regression analysis with weight, height, Tanner stage, calcium intake, and physical activity as determinants with adjustment for age, resulted in a model with Tanner stage in girls and weight in boys for all three BMD-variables. The major independent determinant of BMD was the Tanner stage in girls and weight in boys.

## Introduction

During childhood and adolescence bone mineral density (BMD) increases untill peak bone mass is reached.[1] Peak bone mass and subsequent bone loss are important determinants of osteoporosis later in life.[2] It is essential to know which factors influence BMD in childhood, with the goal of achieving optimal peak bone density.

At present dual energy x-ray absorptiometry is the method of choice to measure BMD because of low radiation exposure, great precision and accuracy.[3]

In the present study the BMDs of the lumbar spine and total body were measured in 500 children and adolescents. Small children were measured with special pediatric software, which is more precise than standard software.[4] The volumetric BMD of the lumbar spine (BMAD) was calculated to correct for bone size. Lumbar spine mainly consists of trabecular bone, while the bone of the total body consists of 80 % cortical bone.[5] BMDs of the lumbar spine and total body increase with age during childhood.[6-11] It is controversial whether volumetric BMD increases with age.[12-15]

The objective of this study was to gain reference values of BMD for healthy Dutch children and to evaluate the influence of age, weight, height, puberty, calcium intake and physical activity on BMD.

## Subjects and Methods

*Subjects*

A total of 500 children and adolescents aged between 4-20 yr of age were examined (205 boys and 295 girls). The ethnicity was Caucasian for 444 children (188 males), black for 21 children (7 males) and Asian for 35 children (9 males). The non-Caucasian children were analyzed as a separate group. The participants were recruited from three primary schools and two secondary schools of the city Rotterdam in cooperation with the Organisation of Child and Adolescent Welfare of Rotterdam. The study protocol was approved by the ethics committee of the University Hospital Rotterdam. Written informed consent was obtained from parents or from subjects older than 16 yr of age.

*Methods*

A questionnaire was administered to all subjects to determine calcium intake, physical activity, vitamin and fluoride use, medical history, smoking, prematurity at birth, low birth weight, previous fractures, menarche, regularity of menstrual periods, use of oral contraceptives and country of birth of both parents during an interview. The questions were asked of one of the parents and in the older children also of themselves. Calcium intake was determined by a detailed food frequency questionnaire of dairy products.[16] Habitual physical activity included physical education classes, organized sports, recreational activity and habitual walking and cycling and was measured in minutes per week.[17] Children who were treated with oral corticosteroids, anticonvulsants or heparin or who suffered from metabolic bone disease, disease of the kidneys, liver, or thyroid, diabetes mellitus, or cystic fibrosis were excluded from the study (n=4). Height was measured with a fixed stadiometer. Weight was measured without shoes on a standard clinical balance.

As validated previously.[18] pubertal development was evaluated by self-assessment of breast and pubic hair stage in girls and genitalia and pubic hair stage in boys, according to the method of Tanner.[19] Subjects were given pictures and written descriptions and selected the picture that most accurately reflected their appearance. When there were discrepancies between the two variables, greater emphasis was placed on the degree of breast development in girls and of genital development in boys for the determinations of Tanner stage.

BMD (grams per $cm^2$) of lumbar spine and total body were measured by dual energy x-ray absorptiometry (DXA, Lunar DPXL/PED, Lunar Radiation Corp., Madison). Pediatric software was used for children with a weight below 30 kg. During measurement of the lumbar spine the child was supine and the physiological lumbar lordosis

was flattened by elevation of the knees. All measurements were performed and analyzed by the same person (A.B.). Quality assurance was performed daily. The coefficient of variation has been reported to be 1.04 % for spine BMD and 0.64 % for total body BMD.[20] The coefficient of variation was not determined because it was considered unethical to measure a child several times. Of 43 children only the BMD of the lumbar spine was measured.

The BMD (grams per $cm^2$) from this measurement is an areal density that varies with bone size. Ancillary DXA-derived data were used to calculate apparent BMAD of the lumbar spine with the model BMAD = BMD x [4 / ($\pi$ x width)]. The lumbar body was assumed to have a cylindrical shape. The validity of this model was tested using in vivo volumetric data obtained from magnetic resonance imaging of lumbar vertebrae.[21]

*Statistical analysis*

The best model for adjustment for age was chosen by multiple regression analysis. Multiple regression analysis was used to determine the association of various factors with BMD. Dummy variables were used for categorical variables that had more than two categories. Two sample t-tests were used to test differences in calcium intake and physical activity between boys and girls.

## Results

The BMD of lumbar spine and total body and lumbar spine BMAD increased with age (Figure 1, Table 1). Due to the small number of subjects between 18-20 yr of age, they were combined in one group in Table 1. During puberty the increment was higher than before puberty. The accumulation started to increase at the age of 11 yr in girls and at the age of 13 yr in boys. The variance increased during puberty. After the age of 16 yr the age-dependent increase in BMD leveled off in girls whereas in boys it continued. Girls had higher lumbar BMD and BMAD than boys at all ages. There was no difference in total body BMD between boys and girls.

The best model for adjustment for age resulted in a model for girls with the factors age, $age^2$ and $age^3$ and for boys with the factors age and $age^2$, for BMD as well as for BMAD. Adjustment for age was performed in this way unless reported otherwise.

The associations of the various factors with BMD and BMAD adjusted for age are listed in Table 2.

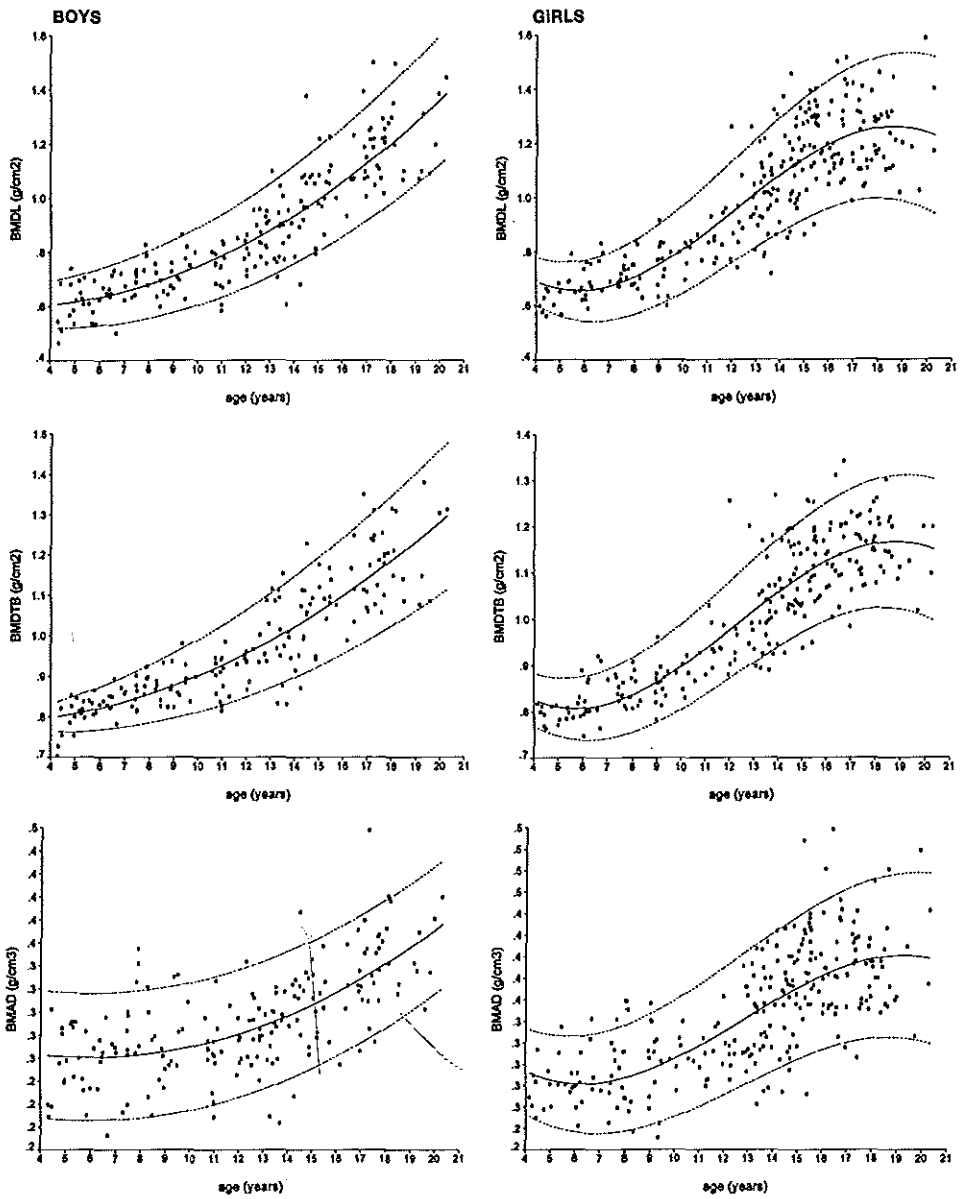


**Figure 1**

*Relation between age and bone mineral density of lumbar spine (BMDL, grams per cm²) and of total body (BMDTB, grams per cm²) and bone mineral apparent density of lumbar spine (BMAD, grams per cm³) in boys and girls. The line showes the best fitted function with the factors age, age² and age³ for girls and age and age³ for boys. The dotted lines represent the 5 % and 90 % prediction limits.*

After adjustment for age, height had a significant positive association with lumbar spine and total body BMD in boys and with lumbar spine BMD in girls. Height had no significant association with BMAD in both sexes, after adjustment for age.
Weight correlated significantly with all three BMD variables after adjustment for age.
Tanner stage had a significant positive association with BMD and BMAD of the lumbar spine and with total body BMD in boys and girls. The increases between the Tanner stages are shown in Table 3.
After adjustment for age, Tanner stage correlated still significantly with lumbar spine BMD and BMAD and total body BMD in girls (respectively $p<0.001$, $p=0.001$ and $p<0.001$). The increases between Tanner stage III to IV and IV to V were significant in girls for all three BMD-variables adjusted for age.
After adjustment for age, Tanner stage correlated significantly with lumbar spine BMD ($p=0.03$), but not with total body BMD or with lumbar spine BMAD in boys.
One hundred and forty-three girls had experienced menarche. Girls of the same age who had experienced menarche had higher lumbar spine and total body BMD and spinal BMAD than girls who had not. To evaluate whether the age at menarche influenced BMD, an analysis was performed of the girls who had experienced menarche with adjustment for age. An earlier age at menarche was associated with a higher BMD (regression coefficient 0.0032 for lumbar spine BMD and 0.0025 for total body BMD; both $p<0.001$). There was no significant association for BMAD. The girls with regular periods had higher total body BMD than girls with irregular periods (regression coefficient 0.043; $p=0.01$) independent of age. For the lumbar spine BMD and BMAD the difference did not reach significance (respectively $p=0.06$ and $p=0.96$).
The mean calcium intake of the Caucasian children was 1180 mg/day (sd 516). There was no significant difference in calcium intake between boys and girls and no significant correlation with age.
Calcium intake had no significant association with BMD in girls. In boys, calcium intake was positively correlated to total body BMD ($p<0.01$) independent of age; the effect did not reach significance for lumbar spine BMD ($p=0.12$).
Calcium intake had no significant association with lumbar spine BMAD.
Physical activity was significantly higher in boys than in girls (mean 9.1 (sd 5.4) vs. 7.5 (SD 4.0) h/week, $p<0.001$). Physical activity had no significant association with BMD and BMAD in girls. In boys physical activity had a significant positive correlation with lumbar spine ($p<0.05$) and total body ($p<0.05$) BMD, after adjustment for age, but not with lumbar spine BMAD.
Ethnicity had no significant association with BMD or BMAD in boys. In girls ethnicity had a significant influence on total body BMD. The girls of Asian ethnicity had a lower total body BMD than the Caucasian girls. The BMD and BMAD of the children

**Table 1**

*Mean Bone Mineral Density values of lumbar spine (BMDL g/cm$^2$), Bone Mineral Apparent Density of lumbar spine (BMAD g/cm$^3$) and Bone Mineral Density of total body (BMDTB g/cm$^2$) and standard deviations (SD) in boys and girls.*

| Age (yr) | n | BMDL | SD | BMAD | SD | n | BMDTB | SD |
|---|---|---|---|---|---|---|---|---|
| BOYS | | | | | | | | |
| 4 - 5 | 9 | 0.591 | 0.09 | 0.250 | 0.03 | 9 | 0.781 | 0.05 |
| 5 - 6 | 12 | 0.625 | 0.06 | 0.261 | 0.03 | 12 | 0.826 | 0.02 |
| 6 - 7 | 10 | 0.656 | 0.07 | 0.260 | 0.03 | 10 | 0.843 | 0.03 |
| 7 - 8 | 13 | 0.720 | 0.06 | 0.282 | 0.04 | 13 | 0.866 | 0.03 |
| 8 - 9 | 11 | 0.685 | 0.05 | 0.261 | 0.03 | 11 | 0.870 | 0.04 |
| 9 - 10 | 8 | 0.755 | 0.08 | 0.288 | 0.03 | 8 | 0.892 | 0.05 |
| 10 - 11 | 11 | 0.726 | 0.08 | 0.257 | 0.03 | 11 | 0.894 | 0.05 |
| 11 - 12 | 9 | 0.791 | 0.07 | 0.282 | 0.03 | 9 | 0.929 | 0.04 |
| 12 - 13 | 18 | 0.846 | 0.08 | 0.282 | 0.03 | 10 | 0.961 | 0.07 |
| 13 - 14 | 17 | 0.868 | 0.13 | 0.280 | 0.03 | 15 | 0.998 | 0.10 |
| 14 - 15 | 16 | 0.977 | 0.17 | 0.306 | 0.04 | 15 | 1.030 | 0.09 |
| 15 - 16 | 12 | 1.058 | 0.10 | 0.312 | 0.03 | 9 | 1.111 | 0.04 |
| 16 - 17 | 11 | 1.119 | 0.12 | 0.319 | 0.03 | 9 | 1.133 | 0.11 |
| 17 - 18 | 19 | 1.204 | 0.11 | 0.347 | 0.04 | 17 | 1.187 | 0.08 |
| 18 - 20 | 12 | 1.238 | 0.16 | 0.355 | 0.03 | 11 | 1.202 | 0.11 |
| GIRLS | | | | | | | | |
| 4 - 5 | 9 | 0.624 | 0.04 | 0.278 | 0.02 | 9 | 0.795 | 0.02 |
| 5 - 6 | 9 | 0.674 | 0.07 | 0.288 | 0.02 | 9 | 0.810 | 0.03 |
| 6 - 7 | 11 | 0.708 | 0.08 | 0.288 | 0.03 | 11 | 0.828 | 0.06 |
| 7 - 8 | 14 | 0.710 | 0.03 | 0.290 | 0.03 | 14 | 0.843 | 0.03 |
| 8 - 9 | 11 | 0.749 | 0.10 | 0.301 | 0.05 | 11 | 0.860 | 0.05 |
| 9 - 10 | 9 | 0.745 | 0.08 | 0.296 | 0.04 | 9 | 0.863 | 0.03 |
| 10 - 11 | 8 | 0.767 | 0.10 | 0.297 | 0.04 | 8 | 0.863 | 0.07 |
| 11 - 12 | 11 | 0.887 | 0.14 | 0.313 | 0.03 | 11 | 0.969 | 0.11 |
| 12 - 13 | 15 | 0.950 | 0.13 | 0.327 | 0.03 | 10 | 0.974 | 0.09 |
| 13 - 14 | 30 | 1.024 | 0.15 | 0.345 | 0.04 | 25 | 1.021 | 0.09 |
| 14 - 15 | 32 | 1.121 | 0.15 | 0.364 | 0.04 | 30 | 1.081 | 0.07 |
| 15 - 16 | 28 | 1.239 | 0.12 | 0.396 | 0.04 | 23 | 1.126 | 0.08 |
| 16 - 17 | 26 | 1.230 | 0.15 | 0.392 | 0.05 | 23 | 1.143 | 0.09 |
| 17 - 18 | 22 | 1.214 | 0.11 | 0.390 | 0.03 | 21 | 1.156 | 0.06 |
| 18 - 20 | 21 | 1.246 | 0.15 | 0.397 | 0.05 | 20 | 1.159 | 0.07 |

**Table 2**

*Association of various factors with lumbar spine bone mineral density (BMDL, g/cm²), total body bone mineral density (BMDTB, g/cm²) and lumbar spine bone mineral apparent density (BMAD, g/cm³), after adjustment for age.*

| | | BOYS | | GIRLS | |
|---|---|---|---|---|---|
| | | regression coefficient 1/1000 | p-value (ns = not significant) | regression coefficient 1/1000 | p-value (ns = not significant) |
| Height | BMDL | 7.04 | < .001 | 3.62 | .002 |
| (cm) | BMDTB | 3.53 | < .001 | 0.79 | ns |
| | BMAD | 0.31 | ns | 0.42 | ns |
| Weight | BMDL | 5.48 | < .001 | 5.73 | < .001 |
| (kg) | BMDTB | 5.40 | < .001 | 5.15 | < .001 |
| | BMAD | 0.63 | .02 | 0.75 | .01 |
| Calcium | BMDL | 2.57 | ns | 1.07 | ns |
| intake | BMDTB | 2.95 | .009 | 0.74 | ns |
| (x 100 mg/day) | BMAD | 0.50 | ns | 0.05 | ns |
| Physical | BMDL | 3.11 | .04 | 0.17 | ns |
| activity | BMDTB | 2.11 | .04 | 0.06 | ns |
| (hour/week) | BMAD | 0.23 | ns | 0.03 | ns |

**Table 3**

*Mean bone mineral density (± SD) of lumbar spine (BMDL) and total body (BMDTB) and bone mineral apparent density (BMAD) per Tanner stage.*

** significant increase compared to previous stage, [1] $p<0.05$, [2] $p<0.01$, [3] $p<0.001$, [4] $p<0.0001$.*

| Tanner stage | BOYS n | BMDL | BMDTB | BMAD |
|---|---|---|---|---|
| I | 79 | 0.69 ± 0.09 | 0.86 ± 0.06 | 0.27 ± 0.03 |
| II | 24 | 0.82 ± 0.10 *[3] | 0.94 ± 0.07 *[3] | 0.28 ± 0.03 |
| III | 14 | 0.91 ± 0.15 *[1] | 1.02 ± 0.10 *[1] | 0.29 ± 0.04 |
| IV | 46 | 1.08 ± 0.17 *[4] | 1.11 ± 0.12 *[2] | 0.32 ± 0.04 *[2] |
| V | 26 | 1.17 ± 0.14 *[2] | 1.15 ± 0.09 *[1] | 0.34 ± 0.04 *[1] |
| | GIRLS | | | |
| I | 67 | 0.71 ± 0.08 | 0.84 ± 0.05 | 0.29 ± 0.03 |
| II | 14 | 0.83 ± 0.07 *[2] | 0.93 ± 0.05 *[3] | 0.30 ± 0.01 |
| III | 21 | 0.96 ± 0.15 *[2] | 0.97 ± 0.08 | 0.33 ± 0.04 |
| IV | 74 | 1.14 ± 0.15 *[4] | 1.08 ± 0.09 *[4] | 0.37 ± 0.04 *[4] |
| V | 79 | 1.22 ± 0.14 *[4] | 1.15 ± 0.08 *[4] | 0.39 ± 0.04 *[3] |

with black ethnicity did not differ from those of the other children.

The children with Asian ethnicity had a significantly lower calcium intake than the Caucasian children (759 vs. 1180 mg/day). The Asian girls had a significantly lower physical activity (4.9 vs. 7.5 h/week) than the Caucasian girls. The physical activity of the Asian boys was not significantly different. The calcium intake and physical activity of black children were not significantly different from those of Caucasian children.

A history of prematurity (n=18) or low birth weight (n=24), smoking (n=32), two or more

fractures in the past (n=22), use of oral contraceptives (n=30) and the use of vitamins (n=126) or fluoride (n=69) were not associated with BMD or BMAD.

Stepwise forward selection in multiple regression analysis with weight, height, Tanner stage, calcium intake, and physical activity as determinants with adjustment for age and with BMD as the dependent variable resulted in a model with weight and Tanner stage for lumbar spine BMD in girls and explained 80 % of the variance. The factors weight and Tanner stage had a significant influence on total body BMD ($r^2$ = 85 %) and the factor Tanner stage had a significant influence on lumbar spine BMAD of girls ($r^2$ = 57

%).

The model for lumbar spine BMD of boys included the factors weight and height ($r^2$ = 85 %). Weight and calcium intake were the factors with a significant influence on total body BMD of boys ($r^2$ = 88 %). The model for BMAD of boys included the factor weight ($r^2$ = 46 %).

## Discussion

In this study determinants of BMD were evaluated in 500 healthy children and adolescents. Weight in boys and Tanner stage in girls had a significant and independent correlation with all three BMD variables.

Determinants of BMD in healthy persons are genetic-ethnic factors, hormonal status, calcium intake, physical activity and weight.

Forty-six to 62 percent of the variance of BMD could be attributed to genetic factors in a study with parents and their children.[22] Twin studies showed a higher heritability, up to 80 %.[23,24] This might be an overestimation as a result of more common lifestyle factors.

Bone density is higher in black than in white subjects and lower in Chinese and Japanese.[25,26] We found a lower total body BMD of girls of Asian ethnicity than of Caucasian girls. The lower bone density in Asians might be attributed to low calcium intake.[26] Calcium supplementation increased the bone mineral content of Chinese children with habitually low calcium intakes.[27]

Our values for lumbar spine BMD are higher than published values of Finnish children [13] and comparable to those of Spanish children [6] measured using DXA equipment from the same manufacturer. There may be geographical differences in BMD. A study in adults found higher incidence rates of hip fractures in the northern part of Europe compared to the rest of Europe.[28]

During puberty there was a large increase in BMD and BMAD. Lumbar spine BMD and BMAD and total body BMD increased significantly with higher Tanner stages, as was previously found for lumbar spine BMD [6,10,29] and total body BMD.[7] During puberty growth hormone as well as sex steroid levels increase and both have a positive influence on BMD.[30,31] The influence of puberty on BMD was higher in girls than in boys. In multiple regression analysis Tanner stage did not correlate significantly with BMD in boys, whereas in girls it was the major determinant. Animals studies showed a more important role of estrogen than of androgen in mineralization of the skeleton.[32] Estrogen is an important determinant of BMD in girls during puberty. This is illustrated by our results showing that girls who had an early menarche or regular periods had

higher BMD. Other studies showed that late menarche and amenorrhea in ballet dancers and patients with anorexia nervosa were related to a reduced BMD and fractures.[33-36] Late puberty and amenorrhoea are risk factors for low BMD in girls.

A few studies showed that persons who consume greater quantities of calcium early in life have greater bone mass later.[37,38] Peak bone mass is optimal when the threshold calcium balance is met.[39] The threshold is the level of calcium intake below which skeletal accumulation of calcium varies with intake and above which it remains constant. According to Matkovic et al.[39] the threshold values are higher than the recommended dietary allowances for calcium (800 mg/day) during childhood and 1200 mg/day during adolescence. The mean calcium intake in our study was 1180 mg/day.

Johnston et al.[40] showed that calcium supplementation (1000 mg calcium/day) enhanced the rate of increase in BMD in prepubertal children. This study was a 3-yr, double blind, placebo-controlled trial in 70 pairs of identical twins. The increase in BMD was twice as high at the radius (cortical bone) as at the lumbar spine (trabecular bone). We also found a higher correlation between calcium intake and total body BMD, which mainly consists of cortical bone, than between calcium intake and lumbar spine BMD in boys.

An adequate calcium intake during childhood is important for optimal mineralization of the skeleton.

Slemenda et al.[41] found that the total hours of weight-bearing activity per week was positively correlated to BMD of the radius and hip in boys and girls 5-14 yr of age. Other studies found a positive correlation between physical activity and lumbar spine BMD[42] or femoral neck BMD[13] in children. In a prospective study it was found that the men and women with the highest levels of exercise at the age of 9-18 yr had higher femoral BMD at the age of 20-29 yr than those with the lowest levels; only the men with the highest levels of exercise had also higher BMD of the lumbar spine.[43] In our study physical activity had a positive association with BMD in boys only. The low variance in physical activity in girls may be the reason why no association was found between physical activity and BMD in girls.

The effect of weight on BMD is due to load on weight-bearing bones[44], comparable to the influence of physical activity.

Children who are underweight and inactive are at risk of developing low BMD.

DXA measures bone mineral content within the projected area; the correction for area removes some, but not all, of the dependence on bone size. To correct completely for bone size we calculated volumetric density for the lumbar spine; this was not possible for the total body measurement. Bone size might be an independent determinant of bone strength.[45,46] Studies showed the relation between areal BMD and both strength[47] and fracture risk[48], which justifies the use of areal BMD. Diagnostic sensitivity was

higher and precision error was lower for BMD than for BMAD in postmenopausal women.[46] Data for the true volumetric density of children are scarce, because of the high radiation dose of quantitative computed tomography. A study of true volumetric spinal bone density measured by quantitative computed tomography showed no increase between 2 and 12 yr in girls.[12] Kröger et al.[13] and Lu et al.[15] found a significant age-dependent increase in calculated lumbar spine BMAD in girls and boys. Our results also showed an increase in BMAD with age.

Height, calcium intake and physical activity had no significant influence on spinal BMAD; therfore, the influence of these variables on spinal BMD could be due to an increase in bone size.

This cross-sectional study provides reference values for lumbar spine and total body BMD of children and adolescents of a West-European country. Lumbar spine and total body BMD and lumbar spine BMAD increase with age with a higher increment during puberty. Determinants of BMD are age, sex, genetic-ethnic factors, hormonal status, calcium intake, physical activity and weight. The major determinant of BMD during childhood appeared to be weight in boys and pubertal development in girls.

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# Chapter 2.2

# Determinants of body composition, measured by dual energy x-ray absorptiometry, in Dutch children and adolescents.

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## Abstract

Knowledge about body composition is important in metabolic and nutritional studies. In this cross-sectional study the body composition of 403 healthy white Dutch children and adolescents was evaluated by using dual energy x-ray absorptiometry (DXA). Possible determinants of body composition were analyzed. In 85 subjects the results of bioelectrical impedance analysis (BIA) were compared with DXA. Fat mass, lean tissue mass and bone mineral content were greater in older boys and girls. Percentage body fat was greater in older girls but not in boys and it was higher in girls than in boys at all ages. From the age of 14 years boys had higher lean tissue mass and bone mineral content than girls. Tanner stage had a significant relation with body composition in both sexes. Percentage body fat was lower in boys in stage 4 than in stage 3 and was higher in consecutive Tanner stages in girls. After adjustment for age, Tanner stage was significantly positively related to lean tissue mass and bone mineral content in boys and girls and to percentage body fat and fat mass in girls. The profession of the parents and the education of the father had a significant negative correlation with percentage body fat and fat mass in girls ($p<0.01$). Physical activity was related to lean tissue mass ($p=0.001$) but not to fat mass in boys after adjustment for age. A high correlation and a small difference was found between lean body mass by BIA and lean tissue mass by DXA. Body composition in healthy Dutch children and adolescents is related to age, sex, Tanner stage, socioeconomic status and physical activity.

## Introduction

For many purposes anthropometric measurements as weight for age and body mass index (BMI; in $kg/m^2$) provide satisfactory information about the nutritional status of children. However, sex, age, ethnicity and diseases cause a considerable variability in body composition that is not reflected in anthropometry. In metabolic and nutritional studies analysis of body composition is required.

In the present study body composition was assessed by dual energy x-ray absorptiometry (DXA). Bioelectrical impedance analysis (BIA) was also performed in a subgroup of subjects. DXA provides precise body composition analysis with a low radiation exposure and is suitable for children.[1] BIA is an indirect method that uses portable equipment. It is inexpensive and relatively easy to perform in children.

Recently, there have been some studies describing body composition in relation to age and sex.[2-4] In the present study the relation of body composition to age, sex, puberty, socioeconomic status, and physical activity is described in Dutch subjects and the re-

sults obtained with DXA and BIA are compared.

## Subjects and methods

*Subjects*

A total of 403 white children and adolescents aged 4 to 20 years were examined by DXA (234 girls and 169 boys). Nonwhite (21 black and 32 Asian children) were excluded because of known racial differences in body composition.[5] The subjects were recruited from three primary schools and one secondary school of the city of Rotterdam in cooperation with the Organisation of Child and Adolescent Welfare of Rotterdam. The schools were selected on the basis of their locations, which were in the neighborhood of the hospital and in different socioeconomic areas of Rotterdam. The children and adolescents were recruited through a standard letter asking them to participate in a study on bone mineral density. No active strategy was used to boost participation rate. Thirty percent of the pupils responded positively. Because of logistic limitations, BIA could be performed in only 85 children (44 girls and 41 boys). This group did not differ with respect to BMI from those not measured with BIA. The study protocol was approved by the Ethics Committee of the University Hospital Rotterdam. Written informed consent was obtained from parents or from subjects older than 16 years of age.

*Methods*

Height was measured with a fixed stadiometer. Weight was measured without shoes on a standard clinical balance. All subjects were interviewed with the help of their parents by using a questionnaire. The physical activity of the participants and occupation and education of both parents were determined. Habitual physical activity included physical education classes, organized sports, recreational activity and habitual walking and cycling and was measured as minutes per week.[6] The Registrar General's Classification[7] was used for the occupation classification of the parents. Six groups were used (1, professional; 2, intermediate; 3, skilled; 4, partly skilled; 5, unskilled; 6, unemployed). The highest classification of either father or mother was used in the analysis. The Dutch education classification into five groups was used.[8] Class 1 corresponds to the most education. For a single-parent family only the profession and education of the parent who took care of the child was used in the analysis. Data concerning the profession of the parents of one girl, the education of the father of two boys and two girls and the education of the mother of two girls are missing.

As validated previously[9] pubertal development was evaluated by self-assessment of

breast and pubic hair stage in girls and genitalia and pubic hair stage in boys according to Tanner.[10] Subjects were given pictures and written descriptions and selected the picture that most accurately reflected their appearance. When there were discrepancies between the two variables, greater emphasis was placed on the degree of breast development in girls and of genital development in boys for the determination of the Tanner stage.

During the interview the participants were asked if they suffered from chronic diseases. Two children who took oral corticosteroidse and one child undergoing chemotherapy because of cancer were excluded.

Body composition was measured by DXA (Lunar DPXL/PED, Lunar Radiation Corporation, Madison, WI). Fat mass, lean tissue mass and bone mineral content are assessed. Total weight measured by DXA is the sum of these three variables. The pediatric medium scan mode was used for children with a weight below 30 kg as recommended by the manufacturer. The fast adult scan mode was used for the other subjects. Daily quality assurance was performed. The coefficients of variation for the pediatric body weight range have been reported 4.1 % for fat mass, 1.0 % for lean tissue mass and 1.8 % for bone mineral content[11] and for the adult weight 2.2 % for fat mass, 1.05 % for lean tissue mass and 0.64 % for bone mineral content.[12] It was considered unethical to perform multiple scans on a child. The bone mineral density results measured by DXA are published separately.[13]

Whole body resistance and reactance were measured in 85 children by using BIA (BIA 101, RJL-systems, Detroit). Current injector electrodes were placed on the dorsal surface of the left hand, just proximal to the phalangeal-metacarpal joints and on the surface of the left foot, just proximal to the transverse arch. Detector electrodes were placed on the posterior side of the left wrist and on the left ankle joint.[14] With these values and age, sex, weight and height lean body mass was calculated by a program provided by the manufacturer with separate programs for children (4 to 12 years), adolescents (13 to 16 years) and adults (17 years and older). Fat mass was calculated as the difference between body weight and lean body mass. All measurements were performed by the same person (AB).

*Statistics*

Logarithmic transformation of percentage body fat, fat mass and bone mineral content was performed because of a skewed distribution. Differences between boys and girls were tested by one year age classes (for example age class 4 was set from the age of 4.00 to 4.99 years) with two sample t tests. Oneway analysis of variance (ANOVA) was used to test differences between Tanner stages. The relation of possible determinants to body composition variables was tested in multiple regression analysis.

Reference centiles of the variables in relation to age were obtained by the method of Royston[15] and Altman.[16] The same adjustment for age was used in the regression analysis. The factors age, age$^2$ and age$^3$ were used for lean tissue mass in both sexes and the factors age and age$^2$ in boys and age, age$^2$ and age$^3$ in girls for the logarithmic transformation of bone mass. The factor age was used for the logarithmic transformation of fat mass and percentage body fat in girls. In boys logarithmic transformation of fat mass (kg) minus 1 was necessary to achieve a normal distribution of the residuals; for the adjustment of age the factor age was used. The logarithmic transformation of percentage body fat in boys was not age dependent. Dummy variables were used for categorical variables.

For comparison between body weight and total tissue by DXA and between lean body mass and percentage body fat by DXA and BIA the limits of agreement of the method of Bland and Altman[17] were used. Pearson correlation coefficients were used to compare variables with a normal distribution.

## Results

Characteristics of the subjects are listed in Table 1. Fat mass, percentage body fat, lean tissue mass and bone mineral content were greater in older boys and girls (Figure 1). The mean percentage body fat was greater in older girls, 24 % compared to 15 %. In boys, the mean percentage body fat was 11 % (95 % confidence interval 9.9 to 11.6) and was not significantly different for boys of different ages. Percentage body fat was greatest in boys aged 11 to 14 years. The mean percentage body fat was significantly higher in girls than in boys at all ages.

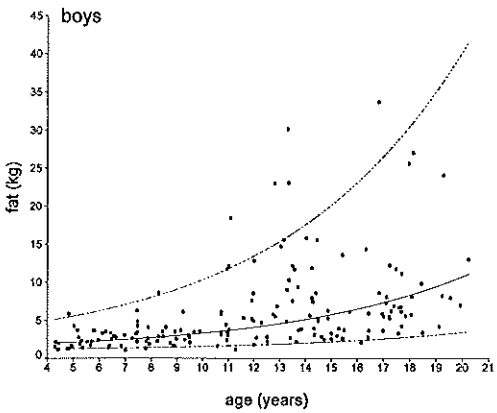
Until the age of 14 years there was no significant difference in lean tissue mass between boys and girls. At older ages the mean lean tissue mass of boys was significantly greater than that of girls. In girls the increase of lean tissue mass was not greater in older girls after the age of 15 years. In boys, lean tissue mass was greater for each older age group until 20 years of age. For children younger than 15 years, there was no significant difference in bone mineral content between boys and girls. At older ages bone mineral content was higher in boys than in girls. There was a strong correlation between lean tissue mass and bone mineral content ($r=0.98$ in boys and $r=0.94$ in girls, both $p<0.001$). The correlation coefficients between lean tissue mass and fat mass were $r=0.48$ in boys and $r=0.61$ in girls and between bone mineral content and fat mass were $r=0.57$ in boys and $r=0.77$ in girls (all $p<0.001$).

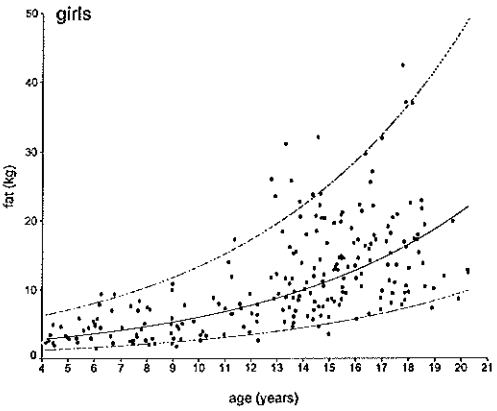
Tanner stage had a significant relation with all the variables of body composition in boys and girls (Table 2).

**Table 1**
*Characteristics of the 403 subjects.*

| | Boys (n=169) | Girls (n=234) |
|---|---|---|
| Occupation parents (n) : | | |
| Class 1 : professional | 44 | 45 |
| Class 2 : intermediate | 50 | 59 |
| Class 3 : skilled | 40 | 71 |
| Class 4 : partly skilled | 14 | 27 |
| Class 5 : unskilled | 11 | 17 |
| Class 6 : unemployed | 10 | 14 |
| Education father / mother (n/n) : | | |
| Class 1 : university | 38 / 19 | 37 / 18 |
| Class 2 : high vocational training | 32 / 35 | 46 / 42 |
| Class 3 : medium vocational training, high or medium secondary education | 37 / 48 | 53 / 65 |
| Class 4 : low vocational training, low secondary education | 32 / 37 | 55 / 73 |
| Class 5 : primary education or less | 22 / 30 | 33 / 34 |
| Physical activity (hrs/week) | 9.0 ± 5.5 | 7.3 ± 3.8 |

In girls percentage body fat and fat mass was higher with higher Tanner stage. In boys, percentage body fat was higher for those between Tanner stages 1 and 3 and lower for those between stage 3 and 5. Lean tissue mass and bone mineral content were significantly higher consecutive Tanner stages in both sexes. After adjustment for age, Tanner stage was still significantly related to lean tissue mass ($p=0.0006$ in boys and $p=0.005$ in girls) and bone mineral content ($p=0.02$ in boys and $p<0.0001$ in girls) and with percentage body fat and fat mass (both $p<0.0001$) in girls. In girls the occupation of the parents was significantly related to age-adjusted percentage body fat ($p=0.008$) and age-adjusted fat mass ($p=0.003$). Percentage body fat and fat mass were higher for children of parents with lower-rank of occupations, except for class 6 (unemployed), after adjustment for age.


boys
fat (kg)
age (years)


girls
fat (kg)
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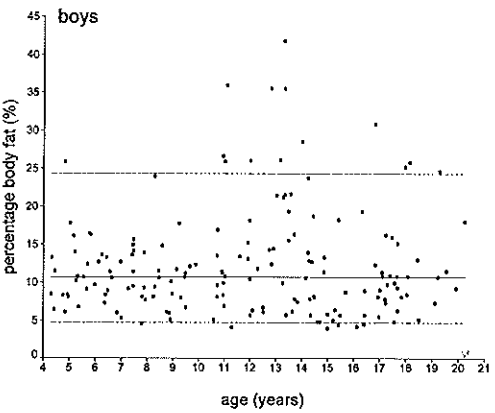
boys
percentage body fat (%)
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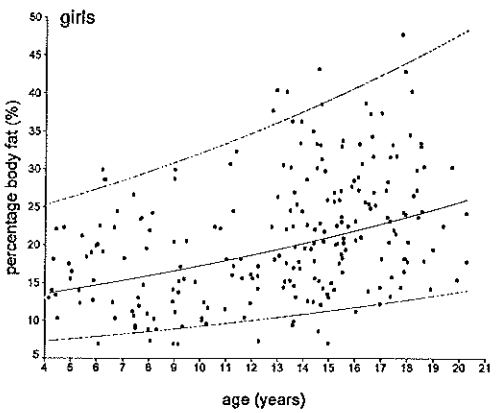
girls
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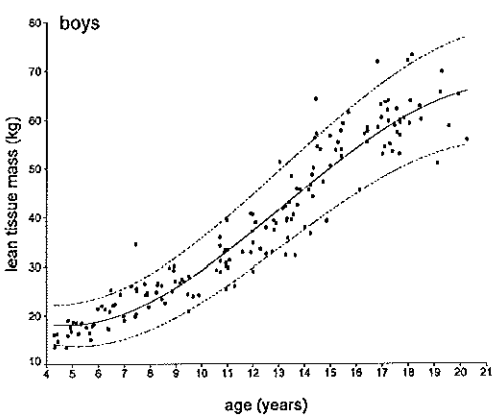
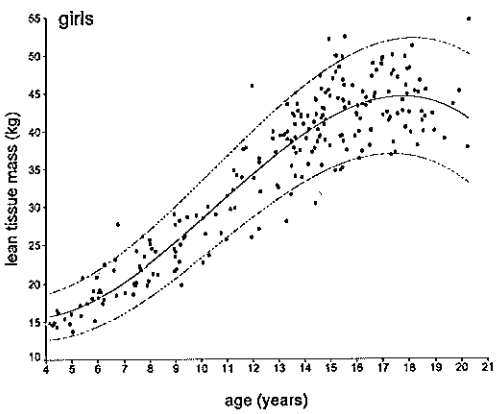

boys
lean tissue mass (kg)
age (years)


girls
lean tissue mass (kg)
age (years)

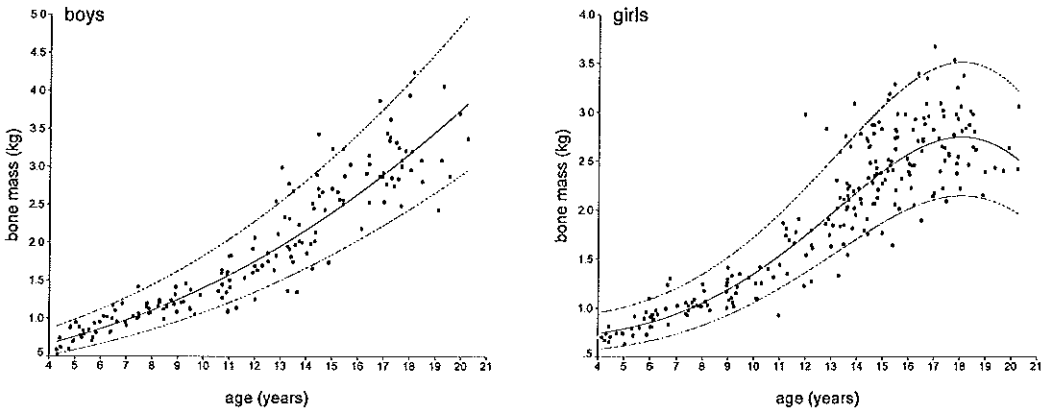



**Figure 1**

*Relation between age and dual energy x-ray absorptiometry-derived fat mass, percentage body fat, lean tissue mass and bone mass in boys and girls. The lines show the best fitted curve. The dotted lines represent the 5 % and 95 % reference centiles.*

**Table 2**

*Percentage body fat, fat mass, lean tissue mass, and bone mineral content by Tanner stage.*

| Tanner stage | n | % body fat | Fat mass (kg) | Lean tissue mass (kg) | Bone mineral content (kg) |
|---|---|---|---|---|---|
| Boys | | | | | |
| 1 | 78 | 10.5 (6.9,15.9) | 1.6 (0.6,4.2) | 24.0 (17.5,30.5) | 1.1 (0.8,1.4) |
| 2 | 18 | 13.4 (7.5,23.9) | 4.6*[4](2.1,10.2) | 35.7*[4](30.4,41.0) | 1.7*[4](1.5,2.0) |
| 3 | 11 | 14.3 (7.0,29.3) | 6.4 (2.2,18.0) | 43.0*[2](34.8,51.1) | 2.2*[1](1.7,2.8) |
| 4 | 39 | 10.1*[1](5.9,17.3) | 5.2 (2.4,11.2) | 53.7*[4](44.7,62.8) | 2.7*[1](2.1,3.4) |
| 5 | 23 | 9.3 (5.8,15.0) | 5.3 (2.6,10.5) | 59.1*[2](53.5,64.8) | 3.0 (2.6,3.4) |
| Girls | | | | | |
| 1 | 68 | 14.8 (10.1,21.7) | 3.9 (2.4,6.2) | 21.4 (16.8,26.1) | 1.0 (0.8,1.2) |
| 2 | 12 | 17.9 (10.8,29.7) | 7.2*[4](3.5,14.7) | 30.7*[4](26.3,35.1) | 1.5*[4](1.2,1.8) |
| 3 | 18 | 18.7 (13.0,27.0) | 8.9 (5.6,14.1) | 36.2*[2](31.4,41.1) | 1.8*[2](1.5,2.2) |
| 4 | 60 | 19.2 (13.6,27.0) | 10.6 (7.0,16.2) | 41.9*[4](37.2,46.6) | 2.3*[4](2.0,2.8) |
| 5 | 76 | 25.5*[4](18.9,34.4) | 15.8*[4](10.5,23.9) | 42.9 (38.7,47.0) | 2.6*[3](2.3,3.0) |

mean, -1 SD, +1 SD in parenthese.

* significant difference with previous Tanner stage

[1] $p<0.05$, [2] $p<0.01$, [3] $p<0.001$, [4] $p<0.0001$

Also the education of the father had a significant negative association with the age-adjusted percentage body fat (p=0.002) and fat mass (p=0.0002) in girls. After adjustment for potential confounders such as Tanner stage and physical activity the relation between the education of the father and body fat was still significant (p=0.03 for age-adjusted fat mass and p=0.01 for age-adjusted percentage body fat), but the relation between the occupations of the parents and body fat was not significant (p=0.06 for age-adjusted fat mass and p=0.08 for age-adjusted percentage body fat). There was no relation between the occupation of the parents or education of the father and lean tissue mass or bone mineral content. The education of the mother had no significant correlation with body composition. In boys occupation and education of the parents had no significant correlation with body composition.

Physical activity had a significant positive correlation with lean tissue mass and bone mineral content in boys, adjusted for age (p=0.0005 and p=0.005 respectively). Physical activity did not correlate with fat mass and percentage body fat. In girls there was no significant correlation between physical activity and the body composition variables.

*Comparison of DXA and other methods*

The mean difference between the total tissue measured by DXA and weight measured by scale was 0.40 kg. The limits of agreement (-2 to 2 SD of the difference) were -1.0 to 1.8 kilograms. BMI correlated significantly with percentage body fat, fat mass, lean tissue mass and bone mineral content measured by DXA. The correlation between BMI and percentage body fat was stronger in girls than in boys (r=0.84 in girls and r=0.56 in boys, both p<0.0001); whereas the correlation coefficient between BMI and fat mass was 0.93 in girls and 0.85 in boys. For the correlation between BMI and lean tissue mass or bone mineral content the correlation coefficients were respectively 0.69 and 0.78 in girls and 0.81 and 0.82 in boys.

The correlation between lean tissue mass measured by DXA and lean body mass measured by BIA was 0.99 (Figure 2A). The mean difference between both measurements was 0.94 kilograms. The limits of agreement were -2.0 to 3.9 kg. The difference increased significantly with higher lean body mass (Figure 2B).

Lean tissue mass measure by DXA is fat free soft tissue. Fat free mass measured by DXA is lean tissue mass plus bone mineral content. The mean difference between fat free mass by DXA and lean body mass by BIA was 2.6 kg (limits of agreement -1.2 to 6.3 kg).

The correlation between percentage body fat measured by DXA and BIA was 0.88. The mean difference in percentage body fat between the two measurements was -5.0 % (limits of agreement -12.3 to 2.3 %), higher values by BIA than by DXA.

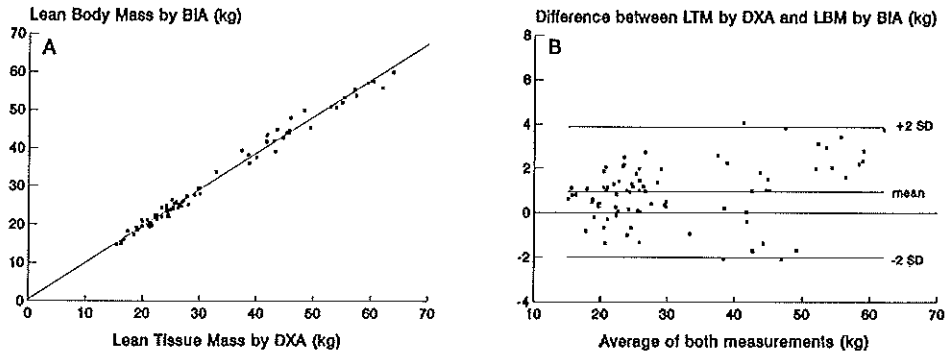



**Figure 2**

*A. Relation between lean tissue mass (LTM) measured by dual energy x-ray absorptiometry (DXA) and lean body mass (LBM) by bio-electrical impedance analysis (BIA). The line represents the regression line.*

*B. Difference between lean tissue mass (LTM) by DXA and lean body mass (LBM) by BIA plotted against the mean for the two methods.*

## Discussion

Body composition in healthy children and adolescents was found to be related to age, sex, Tanner stage, socioeconomic status and physical activity.

Lean tissue mass values were higher in older girls until the age of 15 years whereas in boys the values were higher in successively older groups, confirming previous results.[2,18] Percentage body fat was higher in girls than in boys at all ages, in agreement with other studies.[3,4] Lean tissue mass started to be higher in boys than in girls from the age of 14 years and bone mineral content from the age of 15 years.

Puberty had a significant effect on body composition in boys and girls. In boys the lower percentage body fat and greater lean tissue mass may have been caused by increased growth hormone and androgen concentrations. Synergism between growth hormone and androgens has been described.[19] In boys, plasma growth hormone concentration increases significantly between Tanner stage 3 and 4 and the highest rate of increase is found at Tanner stage 4.[20] In a study in which percentage body fat was measured by underwater weighing, skinfold-thickness measurements and BIA, a decrease in percentage body fat in boys was found between Tanner stage 2 and 3, one stage earlier than in our study.[21] It may be that our subjects overestimated their pubertal stage, although it agrees with the increase of growth hormone levels from

Tanner stage 3 to 4.[20] In other studies of body composition measured by DXA a transient increase in percentage body fat in boys, called the "fat wave", between 10 and 14 years of age was shown.[2,4,22] In these studies there are no data about pubertal stage, but at 10 to 14 years of age the pubertal stage is normally 1 to 3.

The growth hormone secretion rate in girls increases at an earlier pubertal stage than in boys. It starts to increase at stage 2 with the highest rates at stage 3 and IV.[20] Also in girls the increased growth hormone secretion may explain the increase in lean tissue mass.

Socioeconomic status correlated with fat mass and percentage body fat in girls. Class 6, unemployed parents, was an exception. This was a miscellaneous group of parents who were students, disabled, and unemployed. In a study by Gain et al.[23] it was found that children of a lower socioeconomic status had higher percentage body fat during adolescence, probably caused by different dietary habits. Because children with a high percentage body fat have a higher risk of cardiovascular diseases and diabetes[24], health education of adolescents of low socioeconomic status might be a preventive measure.

Physical activity had a positive association with lean tissue mass in boys but did not influence fat mass. Physical activity was significantly lower with a smaller variance in girls than in boys, which may explain why physical activity had no relation with lean tissue mass in girls. DuRant et al.[25] reported that in 4 or 5-year old children physical activity was associated with lower degrees of fatness and more favorable serum lipid and lipoprotein concentrations. In a study of 8-year old children the current physical activity did not influence fatness.[26] In children and adolescents, aged 6 to 17 years, body fat had a weak inverse relation with activity, suggesting that inactivity may not be a primary risk factor in childhood obesity.[27] In the present study, as in most epidemiological studies, a questionnaire about regular activities was used to assess daily physical activity. No information was collected on the intensity of the activities, which may have influenced the results. In children, the reliability of an activity questionnaire improves with age.[28] Especially in young children quantifying physical activity is difficult.

It is possible that the associations with body composition we found are biased by selection (relatively low response rate). However, we consider it unlikely that the response was influenced by determinants of body composition.

DXA, by the manufacturer Lunar used in our study, has been shown to provide precise body composition analysis with a low radiation exposure.[1] There are important differences between the DXA instruments of different manufacturers.[29] DXA (Lunar) was shown to be an accurate method of measuring soft-tissue composition by comparing measurement of pigs in vivo by DXA with chemical analysis after postmortem homogenization.[30, 11] In adults the DXA measurement of body fat (Lunar) was highly

correlated with underwater weighing, skinfold-thickness measurements, and BIA.[31] DXA is able to detect small changes in body composition.[32] The mean difference between weight measured by scale and the total tissue measured by DXA was less than what was found in other studies.[2, 31] The difference may be explained by the fact that the subjects of these studies were older than those in our study and had a higher mean weight.

BMI is used in clinical practice as a measure of nutritional status. BMI correlates with fat mass and lean tissue mass but cannot differentiate between these components. Lean body mass measured by BIA differed less from lean soft tissue mass by DXA than from fat free mass by DXA, which includes bone mineral content. Because fat mass by BIA is calculated as the difference between weight and lean body mass, fat mass and percentage body fat are overestimated if DXA is taken as the standard. Also other studies found a higher fat content with BIA than with DXA of Lunar.[31,33] However, in a study with a DXA of Hologic (QDR-2000, Waltham,MA) the fat mass measured by BIA was lower than by DXA[34], using the same equations to calculate lean body mass by BIA as in our study. Reilly et al.[35] showed that the estimate of lean body mass using BIA depends on which equation is used and can differ up to 3 kg. Published BIA equations for children are cross-validated with hydrodensitometry[36,37] or deuterium dilution space[37] --both of which can be difficult to apply in children-- and $^{40}K$ spectometry.[38] These studies were performed in different age groups and populations or used BIA equipment of different manufacturers. We chose to use the commercial equation developed in Western Europe with separate programs for different age groups. DXA is preferred for a reliable estimation of body composition because it is a precise and accurate method, as shown by studies in adults and animals. However, the advantage of BIA is that it is inexpensive, less time-consuming and uses portable equipment.

In conclusion, puberty has a large influence on body composition in boys and girls. Socioeconomic status was significantly correlated with body fat in girls. Physical activity related to age-adjusted lean tissue mass in boys.

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# Chapter 3

# Bone mineral density and body composition before and during treatment with gonadotrophin-releasing hormone agonist in children with central precocious and early puberty

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## Abstract

Major changes in bone mineral density (BMD) and body composition occur during puberty. In the present study we evaluated BMD, calculated volumetric BMD (BMAD), bone metabolism and body composition of children (32 girls and 2 boys) with central precocious and early puberty (CPP) before and during treatment with gonadotrophin-releasing hormone agonist (GnRH). Patients were studied at baseline and during treatment for 6 months (n=34), 1 year (n=33) and 2 years (n=16). Lumbar spine and total body BMD and body composition were measured with dual energy X-ray absorptiometry. The variables were compared with age- and sex-matched reference values of the same population and expressed as standard deviation score (SDS). Bone age was assessed. Serum calcium, phosphate, alkaline phosphatase, osteocalcin, the carboxy terminal propeptide of type I collagen (PICP), cross-linked telopeptide of collagen I (ICTP), 1,25 dihydroxy vitamin D and urinary hydroxyproline/creatinine and calcium/creatinine ratios were measured.

Mean lumbar spine BMD SDS was significantly higher than zero at baseline ($p<0.02$) and did not differ from normal after two years of treatment. Mean spinal BMAD SDS and total body BMD SDS were not significantly different from zero at baseline and had not changed significantly after two years of treatment. During therapy, fat mass and percentage body fat SDS increased, while lean tissue mass SDS decreased. Mean lumbar spine BMD and BMAD and total body BMD SDS calculated for bone age were all lower than zero at baseline (BMD $p<0.001$, BMAD $p<0.05$) and also after 2 years treatment (respectively $p<0.001$, $p<0.05$ and $p<0.01$). Biochemical bone parameters were significantly higher than prepubertal values at baseline and decreased during treatment. In conclusion, patients with CPP had normal BMD for chronological age but low BMD for bone age after two years of treatment with GnRH. Bone turnover decreased during treatment. Changes in body composition resembled those seen in patients with growth hormone deficiency.

## Introduction

During puberty, bone mineral density (BMD) and height increase and body composition changes markedl.[1-4] In central precocious puberty the hypothalamus-pituitary-gonadal axis is activated before the age of 8 years in girls and before the age of 9 years in boys. Precocious puberty is associated with premature and rapid skeletal maturation leading to decreased final height compared to target height in most patient.[5,6] Gonadotrophin-releasing hormone agonist (GnRH) can be supplied to arrest pubertal development to

improve final height and avoid psychosocial problems.[7-9] In children with central early puberty, final adult height may be slightly improved with GnRH treatment.[9] Treatment with GnRH causes a decline in gonadal sex steroids which may affect BMD, bone metabolism and body composition. Some studies in children with central precocious puberty showed a decrease of BMD after 6 and 12 months of treatment with GnRH [10,11], another study reported no change during treatment.[12] In women with endometriosis and in elderly men with benign prostatic hyperplasia BMD decreased and biochemical markers of bone turnover increased during treatment with GnRH.[13,14]

The aim of the present study was to investigate BMD, bone metabolism and body composition of children with central precocious or early puberty (CPP) before and during treatment with GnRH.

## Patients and methods

*Patients*

At diagnosis all patients had a history of increased growth velocity, girls had breast development Tanner stage 2 or more and boys genital development Tanner stage 2 or more and testis volume 4 ml or more, bone age was advanced more than 1 year beyond chronological age, and a GnRH-stimulated serum luteinizing hormone concentration greater than 10 IU/l. Thirty-four patients participated in the study. Twenty-three girls and 2 boys had true idiopathic central precocious puberty. Seven girls had idiopathic central early puberty: in 3 girls the appearance of pubertal signs started before the age of 9 years and in 4 girls before the age of 10 years. Two girls had organic CPP: one had a meningomyelocele and the other a hydrocephalus with a start of puberty before the age of respectively 9 and 8 years. Mean age at start of treatment was 8.1 years (range 2.8 to 10.8). All patients received therapy with depot leuprolide-acetate 3.75 mg (Lucrin$^{R}$ depot, Abbott) given subcutaneously every 4 weeks. During the first month it was given every two weeks. Puberty suppresion was evaluated by clinical evaluation, repeating GnRH stimulation test after 3 months, by measuring basal serum levels of luteinizing hormone, follicle stimulating hormone and estradiol/testosterone every 6 months, and bone age assessment. All children had complete suppression of plasma concentrations of luteinizing hormone and follicle stimulating hormone during the GnRH test after 3 months of treatment (levels below 5 IU/l). All children except one girl had basal sex steroids concentrations equal or less than prepubertal levels during treatment (estradiol below 50 pmol/l, testosterone below 1 nmol/l). In the girl with incomplete suppression the dose of leuprolide-acetate was doubled. In the first year the mean ratio of the change of ($\Delta$) bone age and $\Delta$ chronological age was 0.72 and in the second year 0.47.

Thirty-four patients had baseline and half year measurements, 33 of them 1 year and 16 children 2 years follow-up. One child stopped treatment after 6 months and one after one year. The other children are still included in this ongoing study. No BMD standard deviation for chronological age could be calculated of one girl with the age of 2.8 years because our reference data start from the age of 4 years. Her data could be used in the evaluation of BMD standard deviation calculated for bone age.

*Methods*

Anthropometry, BMD and body composition measurements and assessment of biochemical bone parameters were performed at baseline and during GnRH treatment for 6 months, 1 year and 2 years. Height was measured with a Harpenden stadiometer. Height was compared to age- and sex-matched reference values[15] and expressed as standard deviation scores (SDS). Body mass index was calculated as weight/(height)$^2$ (kg/m$^2$) and compared to age- and sex-matched reference value[16] and expressed as SDS. Pubertal development was determined according to Tanner.[17]

BMD (g/cm$^2$) of the lumbar spine and total body was measured by Dual Energy X-ray Absorptiometry (DXA) (Lunar, DPXL/PED, Lunar Radiation Corporation, Madison, Wisconson, USA). The coefficient of variation has been reported as 1.04 % for lumbar spine and 0.64 % for total bod.[18] The coefficient of variation (sd) for lumbar spine in our setting is 1.1 (0.2) %. Ancillary DXA-derived data were used to calculate lumbar spine volumetric BMD (bone mineral apparent density BMAD) with the model BMAD = BMD x [4/ ($\pi$ x width)], as validated before.[19] BMD and BMAD results were compared to our age- and sex-matched Dutch reference values[2] and expressed as SDS. With the total body measurement by DXA the body composition was measured as lean tissue mass, fat mass and bone mineral content. The coefficients of variation have been reported as 2.2 % for fat mass, 1.1 % for lean tissue mass and 0.6 % for bone mineral content.[18] Bone mineral content, lean tissue mass, fat mass and percentage body fat were compared to our age- and sex-matched Dutch reference values and expressed as SDS.[3]

Bone age was assessed by one investigator using an X-ray of the left hand according to the Greulich and Pyle method[20] at baseline in 34 patients, after 6 months in 21 patients, after 1 year in 32 patients and after 2 years in 16 patients.

Blood samples were taken for the assessment of calcium, phosphate, alkaline phosphatase, 1,25 dihydroxyvitamin D, osteocalcin, the carboxy terminal propeptide of type I collagen (PICP) and cross-linked telopeptide of collagen I (ICTP). Samples missed of two patients at baseline. One year's results missed in two other patients. Osteocalcin was measured by radioimmunoassay (Incstar Corporation, Stillwater, USA); 1,25 dihydroxyvitamin D by radioimmuno assay of Immuno Diagnostic Systems (Boldon, United Kingdom). PICP and ICTP were measured with a radioimmunoassay kit (Orion Diagnostica, Espoo, Finland).

Our own reference values of prepubertal healthy children for osteocalcin, PICP and ICTP (respectively n=25, n=82 and n=88) were used. In the first morning void of urine the ratio of hydroxyproline and creatinine (OHP/CR) and the ratio of calcium and creatinine (CA/CR) were evaluated. Reference values of Wolthers et al. were used for OHP/CR.[21] Luteinizing hormone and follicle stimulating hormone were assessed by radioimmunoassay (Medgenix, Belgium); estradiol and testosterone by radioimmunoassay of Orion Diagnostica, Espoo, Finland.

*Statistical analysis*

One sample t-tests were performed to compare the mean SDS values to normal. We tested if the average within patient change differed from zero with one sample t-test. Pearson correlation coefficient was calculated to test the association between two variables with a normal distribution. Spearman's rank correlation coefficient was utilized in case of a non-normal distribution.

## Results

The results of BMD, BMAD, body composition, height and body mass index before and during GnRH treatment are shown in Table 1.

At baseline, mean lumbar spine BMD SDS was significantly higher than zero, which is the mean SDS of age-and sex-matched healthy controls. Lumbar spine BMD SDS increased during the first 6 months of therapy and decreased between 6 months and 1 year of treatment ($p<0.01$). After two years, lumbar spine BMD SDS was not significantly different from normal. Mean lumbar spine BMAD SDS and total body BMD SDS were not significantly different from normal at baseline. Lumbar spine BMAD SDS showed a transient increase after 6 months of treatment. Total body BMD SDS remained stable during treatment.Mean total body bone mineral content SDS, lean tissue mass SDS, fat mass SDS and percentage body fat SDS were significantly higher than zero at baseline. Total body bone mineral content SDS had increased after 6 months and one year of treatment compared to baseline. Lean tissue mass SDS decreased significantly during treatment while fat mass SDS and percentage body fat SDS increased .

Mean height SDS and body mass index SDS were higher than zero at baseline. Mean height SDS had decreased after 2 years of treatment. Body mass index SDS increased during treatment.

The two boys did not have lumbar spine BMD SDS higher than zero at baseline. During treatment, all the variables showed the same pattern as seen in the girls.

**Table 1**

*Mean (sd) of variables at baseline and during treatment with GnRH (Rx) in children with CPP. BMD=bone mineral density (g/cm²), BMAD=bone mineral apparent density (g/cm³), BMC=bone mineral content (g), SDS= standard deviation score. The mean SDS was compared with normal and the within patient change from baseline was tested.*

| | baseline | ½ year Rx | 1 year Rx | 2 years Rx |
|---|---|---|---|---|
| | n=33 | n=33 | n=32 | n=16 |
| lumbar spine BMD SDS | 0.51 (1.14)[3] | 0.72 (1.04)[1,b] | 0.53 (1.04)[3] | 0.11 (0.81) |
| lumbar spine BMAD SDS | 0.18 (1.21) | 0.42 (1.10)[4,c] | 0.30 (1.24) | -0.01 (1.04) |
| total body BMD SDS | 0.09 (1.21) | 0.24 (1.27) | 0.35 (1.08) | 0.16 (0.72) |
| total body BMC SDS | 0.60 (1.17)[2] | 0.83 (1.13)[1,c] | 0.88 (1.17)[1,d] | 0.70 (1.17)[4] |
| lean tissue mass SDS | 0.91 (1.19)[1] | 0.74 (1.10)[1,b] | 0.60 (1.18)[2,b] | 0.33 (1.27)[b] |
| fat mass SDS | 0.38 (0.89)[4] | 0.76 (0.94)[1,a] | 0.98 (0.99)[1,a] | 1.02 (1.13)[2,b] |
| % body fat SDS | 0.44 (1.09)[4] | 0.96 (1.15)[1,a] | 1.24 (1.09)[1,a] | 1.39 (1.36)[1,a] |
| height SDS | 1.08 (1.20)[1] | 1.09 (1.15)[1] | 0.97 (1.19)[1] | 0.77 (1.21)[4,d] |
| body mass index SDS | 0.96 (1.12)[1] | 1.16 (1.12)[1,c] | 1.38 (1.11)[1,a] | 1.37 (1.21)[1,c] |

[1] $p<0.001$, [2] $p<0.01$, [3] $p<0.02$, [4] $p<0.05$ higher than zero

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.02$, [d] $p<0.05$ compared to baseline

If SDS was calculated for bone age instead of chronological age ($SDS_{BA}$) mean lumbar spine BMD $SDS_{BA}$ was -0.82 (sd 0.91), mean BMAD $SDS_{BA}$ was -0.46 (sd 1.11) and mean total body BMD $SDS_{BA}$ was -1.07 (sd 1.05) at baseline, all significantly lower than zero (lumbar spine and total body BMD $p<0.001$, BMAD $p<0.05$). Mean lumbar spine BMD and BMAD $SDS_{BA}$ and total body BMD $SDS_{BA}$ after two years treatment were still significantly lower than zero (lumbar spine BMD $p<0.001$, total body BMD $p<0.01$, BMAD $p<0.05$) and did not differ significantly from baseline.

The results of biochemical markers of bone metabolism are shown in Table 2. Mean osteocalcin, PICP and ICTP at baseline were significantly higher than those of prepubertal controls (all $p<0.001$). These values and alkaline phosphatase had decreased after 6 months. Mean PICP and ICTP at 6 months and osteocalcin at 12 months were not significantly different from those of prepubertal controls. Urine CA/CR had increased after 6 months, OHP/CR had diminished after 1 year. Serum calcium and phosphate were normal at baseline and did not change significantly during time.

At baseline, lumbar spine BMD SDS correlated with lean tissue mass SDS ($r=0.46$, $p<0.01$) and body mass index SDS ($r=0.46$, $p<0.01$). Total body BMD SDS correlated

with fat mass SDS (r=0.44, p<0.02), percentage body fat SDS (r=0.37, p<0.02) and body mass index SDS (r=0.42, p<0.02). Height SDS, Tanner stage or biochemical bone parameters were not related to lumbar spine BMD or BMAD or total body BMD SDS. Height SDS correlated with lean tissue mass SDS (r=0.76, p<0.001) and total body bone mineral content SDS (r=0.58, p<0.001).

The Δ between baseline and 2 years treatment of height SDS had a correlation with Δ lumbar spine BMD SDS (r=0.57, p<0.05), Δ lean tissue mass SDS (r=0.77, p<0.001) and Δ bone mineral content SDS (r=0.69, p<0.01) and not with Δ total body BMD SDS or Δ lumbar spine BMAD SDS. The change in levels of biochemical markers of bone turnover did not correlate with the change of BMD or BMAD SDS or height SDS.

**Table 2**

*Mean (sd) of biochemical parameters at baseline and during GnRHa (Rx) in children with CPP. PICP=carboxy terminal propeptide of type I collagen; ICTP=cross-linked telopeptide of type I collagen; alk. phosphatase=alkaline phosphatase; 1,25 OHD =1,25 dihydroxyvitamin D; OHP/CR and CA/CR =hydroxyprolin/creatinine and calcium/creatinine (mmol/l per mmol/l) ratio's in first morning void of urine. The mean within patient change from baseline was tested.*

| | baseline | ½ year Rx | 1 year Rx | 2 years Rx |
|---|---|---|---|---|
| | n=32 | n=32 | n=30 | n=16 |
| osteocalcin (µg/l) | 23.5 (7.0) | 19.0 (5.3)[b] | 15.7 (3.8)[a] | 16.3 (3.3)[a] |
| PICP (µg/l) | 494 (190) | 321 (141)[a] | 335 (123)[a] | 276 (84)[a] |
| ICTP (µg/l) | 18.3 (4.0) | 12.8 (2.5)[a] | 12.0 (4.3)[a] | 11.5 (2.6)[a] |
| alk. phosphatase (U/l) | 291 (73) | 217 (47)[a] | 221 (53)[a] | 205 (55)[a] |
| 1,25 OHD (pmol/l) | 136 (47) | 129 (52) | 119 (42) | 123 (28) |
| Urine OHP/CR (mg/g) | 122 (56) | 107 (115) | 76 (31)[b] | 67 (27) |
| Urine CA/CR | 0.20 (0.13) | 0.40 (0.28)[c] | 0.25 (0.16) | 0.31 (0.18)[b] |

[a] p<0.001, [b] p<0.01, [c] p<0.02, [d] p<0.05 compared to baseline

## Discussion

In children with CPP, mean lumbar spine BMD was high, and spinal BMAD and total body BMD were normal for chronological age. After two years of treatment with GnRH mean lumbar spine BMD and BMAD and total body BMD were normal. Lumbar spine BMD and BMAD and total body BMD for bone age were low, before and after two years

of treatment with GnRH. During treatment fat mass and percentage body fat SDS increased, while lean tissue mass SDS decreased. Biochemical markers of bone formation as well as of bone resorption decreased.

Previous studies reported increased lumbar spine (measured with DXA) or radius (measured with single photon absorptometry) BMD for chronological age, but appropiate for bone age in girls with CPP.[10-12] The higher spinal BMD at baseline is in agreement with our findings but we found decreased BMD for bone age. The discrepancy may be caused by differences in reference values, differences in assessment of bone age or differences in timing of start of treatment. In the present study reference values of a large cohort of healthy children of the same population measured on the same DXA apparatus were used.

Mean spinal BMAD (corrected for estimated bone volume) was not increased. BMD is an areal density and does not adjust for bone size completely. Therefore, the high spinal BMD could be due to an increase of bone size.

During puberty estrogens as well as growth hormone (GH) play an important role in bone mineralization and bone metabolism in girls. In early puberty, low levels of estradiol stimulates growth and GH production. A significant increase of GH is seen during early puberty with maximal levels at stage III in girls.[22] Bone modeling of new bone and bone remodeling of existing mineralized tissue are each ongoing processes in growing children. The biochemical markers are not specific for either the process of bone modeling or remodelling.[23] Markers of bone metabolism are related to growth velocity and increase maximally during midpuberty.[24,25] From stage III to V, late puberty, estradiol levels increase significantly, GH levels decrease and markers of bone formation as well as markers of bone resorption decrease.[26] In postmenopausal women and in adults treated with GnRH, the decline of estrogens is associated with an increase of bone turnover, the opposite of what happens during late puberty.[13,14] At baseline, the patients had bone turnover comparable with that of adolescents in early puberty. Bone formation and bone resorption markers were higher than prepubertal values, probably due to the increased growth related with early puberty. The markers of bone turnover decreased to prepubertal levels during treatment. This mainly reflects a decrease in bone modeling.

Increased GH and/or estrogens levels associated with early puberty may have caused the increase of lumbar spine BMD at baseline, which was only found in girls. Lumbar spine BMD was not high in the boys but their number was very limited in our study. However, also in healthy adolescents the influence of puberty on BMD is higher in girls than in boys.[2] Estrogens seems to have a more important role in bone mineralization than androgens.[27] Lumbar spine consists of more trabecular bone than bone of the total body, which is for 80 % cortical bone.[28] Bone turnover is higher in trabecular bone[29] which may explain the differences found between lumbar spine and total body BMD. Also in

postmenopausal women change in BMD is faster of trabecular bone than of cortical bone.[30] The initial increase of lumbar spine BMD and BMAD SDS may be explained by incomplete suppression of puberty during the first months. The decrease in spinal BMD thereafter is probably caused by the decline of estrogens and GH. A decrease in nocturnal GH secretion and subnormal response to GH stimulation tests were described after 3 to 12 months treatment with GnRH in children with CPP.[10,31-33] However, no relation was found between the subnormal GH levels and growth velocity during treatment with GnRH.[33] The relative state of GH deficiency during treatment may explain the decrease of lean tissue mass SDS and the increase of fat mass and percentage fat SDS. GH is known to have lipolytic and anabolic effects. Children and adults with growth hormone deficiency have decreased lean tissue mass and increased fat mass which improve during treatment.[34-37]

In conclusion, children with CPP have normal BMD for chronological age but decreased for bone age after two years of treatment with GnRH. Puberty is an important period for bone accretion. In patients with CPP pubertal development is temporarily inhibited by GnRH. It is unknown if BMD increases normally after cessation of GnRH and if the patients reach a normal peak bone mass. Long-term longitudinal studies till peak bone mass are needed to evaluate BMD and body composition after cessation of treatment.

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# Chapter 4

# Bone mineral density and nutritional status in children with chronic inflammatory bowel disease

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## Abstract

*Background* - Osteoporosis has been reported in adult patients with inflammatory bowel disease.
*Aim* - To evaluate bone mineral density (BMD), nutritional status and determinants of BMD in children with inflammatory bowel disease.
*Patients* - Fifty-five patients (34 boys and 21 girls), 4 to 18 years old. 22 children suffered from Crohn's disease and 33 children from ulcerative colitis.
*Methods* - Lumbar spine and total body bone mineral density (BMD) and body composition were assessed by Dual Energy X-ray Absorptiometry (DXA). The results were expressed as standard deviation scores (SDS). Lean body mass was also assessed by Bio-electrical impedance analysis (BIA). Yearly measurements during two years were performed in 21 patients.
*Results* - The mean SDS of lumbar spine BMD and total body BMD were significantly lower than normal (-0.75 and -0.95, both $p<0.001$). Also height SDS and body mass index SDS were decreased. The decrease in BMD SDS could not be explained by delay in bone maturation. The cumulative dose of prednisone correlated negatively with lumbar spine BMD SDS ($r=-0.32$, $p<0.02$). Body mass index SDS correlated positively with total body BMD SDS ($r=0.36$, $p<0.02$). Patients with Crohn's disease had significantly lower lumbar spine and total body BMD SDS than patients with ulcerative colitis, even after adjustment for cumulative dose of prednisone. In the longitudinal data cumulative dose of prednisone between the measurements correlated negatively with the change in lumbar spine and total body BMD SDS. Lean tissue mass measured by DXA had a strong correlation with lean body mass measured by BIA ($r=0.98$).
*Conclusions* - Children with inflammatory bowel disease have a decreased BMD. Children with Crohn's disease have a higher risk to develop osteopenia than children with ulcerative colitis. Corticosteroid therapy and nutritional status are important determinants of BMD in these patients.

## Introduction

Several studies have reported an increased prevalence of osteoporosis in adult patients with chronic inflammatory bowel disease (IBD).[1-3] An increased rate of spinal bone loss was found in 54 % of adult patients with IBD.[4]
Retardation of growth and skeletal maturation are common in children with IBD.[2,5,6] Little is known about bone mineral density (BMD) of children with IBD. Children with osteopenia have a higher risk on fractures in childhood and also in adult age because of

not reaching their optimal peak bone mass.

Since a few years it is possible to measure quantitatively BMD by Dual Energy X-ray Absorptiometry (DXA). It has a short scan time and low radiation dose which makes it suitable for children.[7] Measurement of BMD of lumbar spine and of total body can be performed. With the total body measurement, body composition in lean tissue mass, fat mass and bone mineral content is assessed.

In the present study with cross-sectional and longitudinal data we investigated the prevalence of and risk factors for a low BMD in children and adolescents with Crohn's disease or ulcerative colitis. The results of body composition measurements by DXA were compared with those obtained by bioelectrical impedance analysis.

## Patients and methods

*Patients*

Fifty-five patients (34 boys and 21 girls) with chronic inflammatory disease were studied. Twenty-two patients suffered from Crohn's disease and thirty-three patients from ulcerative colitis. Diagnosis was made according to the Dutch children's IBD consensus guidelines.[8] The mean age was 13 years (range 4 to 18 years). Thirty-six patients were measured two times, and twenty-one patients three times with intervals of about one year. Fourteen patients had Crohn's disease of the small bowel, four of the colon and four of both. Five patients underwent an ileocecal resection before the first measurement. Two patients with ulcerative colitis suffered also from sclerosing pericholangitis and one patient with ulcerative colitis from chronic active hepatitis. The duration of the symptoms of the disease ranged from one month to 12 years (median duration was 2.2 years).

The total lifetime cumulative dose of prednisone (milligrams) was calculated at the first measurement and also the cumulative dose between the yearly measurements. Twenty patients (36 %) had not been treated with corticosteroids before the first measurement. Three of these received corticosteroids before the second measurement. All patients had been treated with sulphasalazine or mesalazine.

*Methods*

Height was measured with a Harpenden stadiometer. Height was compared to age- and sex-matched reference values[9] and expressed as standard deviation scores (SDS). Weight was assessed by a standard clinical balance. The body mass index was calculated as weight/(height)$^2$ (kg/m$^2$) and compared to age- and sex-matched reference values[10] and expressed as SDS.

Pubertal development was determined according to Tanner.[11] For patients in puberty, delay in puberty was calculated by comparison of Tanner stage and age of the patients with reference data of Dutch children.[9] The delay was calculated as the difference between the age of the patient and the median age of next Tanner stage of the reference group.

BMD of the lumbar spine and total body was measured by Dual Energy X-ray Absorptiometry (Lunar, DPXL/PED, Lunar Radiation Corporation, Madison, Wisconson, USA). BMD results were compared to our age- and sex-matched Dutch reference values (n=500)[12] and expressed as standard deviation scores (SDS). Patients with a BMD SDS below -1.5 were given supplements of calcium 500 mg per day and vitamin D 400 units per day.

With the total body measurement by DXA the body composition was measured as lean tissue mass, fat mass and bone mineral content. Total tissue mass is the sum of these three variables. Percentage body fat is given for total tissue mass. Bone mineral content, lean tissue mass, fat mass and percentage body fat were compared to our age- and sex-matched Dutch reference values and expressed as SDS.[13]

Lean body mass was also estimated with bioelectrical impedance analysis (BIA 101, RJL-systems, Detroit, MI). Two skin electrodes were placed on the dorsal surface of the left hand and wrist, and two electrodes on the surface of the left foot and ankle joint, according to the standard procedure as described before.[14] Whole body resistance and reactance were measured. With these values and age, sex, weight and height the lean body mass was calculated by a programme provided by the manufacturer with separate programmes for children (4 to 12 years), adolescents (13 to 16 years) and adults (17 years and older). Hundred and three measurements with BIA were compared to simultaneous DXA-measurements.

Dietary calcium intake and total caloric intake were assessed in 36 patients by a dietician using a food intake diary of three days. The results were compared to the recommended Dutch intake of calcium and calories per sex and agegroup.

During an interview children above 10 years of age were asked about their daily habitual physical activity[15], which included physical education classes, organized sports, recreational activity and habitual walking and cycling. The results were compared to the healthy controls of the BMD reference population[12] in which the same questionnaire was used.

Bone age was assessed in 52 children by one investigator (AB) using an X-ray of the left hand according to the Tanner-Whitehouse Radius-Ulna-Short bones (RUS) method.[16] Two X-rays of the hand were taken in 30 patients and three X-rays in 14 patients with intervals of about one year.

Blood samples were taken at the first measurement for the assessment of calcium,

phosphate, alkaline phosphatase, parathyroid hormone, 25 hydroxyvitamin D, 1,25 dihydroxyvitamin D, osteocalcin, the carboxy terminal propeptide of type I collagen (PICP) and Insulin-like growth factor 1 (IGF-I). Serum intact parathyroid hormone was determined in 21 patients by radioimmunoassay (Nichols Institute, San Juan Capistrano, CA, USA). 25 hydroxyvitamin D was assessed in 42 patients and 1,25 dihydroxyvitamin D in 23 patients. Osteocalcin and 25 hydroxyvitamin D were measured by radioimmunoassay (Incstar Corporation, Stillwater, USA), and 1,25 dihydroxyvitamin D by radioimmunoassay of Immuno Diagnostic Systems (Boldon, United Kingdom). PICP was measured with a radioimmunoassay kit (Orion Diagnostica, Espoo, Finland). Osteocalcin was assessed in 41 patients and PICP in 39 patients. Our own reference values for osteocalcin and PICP (respectively n=25 and n=82) were used for prepubertal children. Reference values for the older children were subtracted from other studies which used the same assays.[17-19] For measurements of IGF-I (nmol/l) kits of Med-Genix Diagnostics, Fleurus, Belgium were used. IGF-I was assessed in 50 patients. IGF-I sex- and age-matched reference values were based on 600 samples of a healthy Dutch population.[20] In 24-hours urine the ratio of hydroxyproline and creatinine was evaluated in 47 patients and compared to reference values.[21]

*Statistics*

One sample t-tests were performed to compare the mean SDS values to normal. Two sample t-tests were used to compare variables with a normal distribution between two groups. Pearson correlation coefficient was calculated to test the association between two variables with a normal distribution. Spearman's rank correlation coefficient was utilized in case of a non-normal distribution. Multiple regression analysis was used for adjustment of confounders and to test possible pathogenetic factors of osteopenia simultaneously. One-way analysis of variance was performed to test difference in more than two groups.

## Results

The mean SDS of the lumbar spine BMD at the first measurement was -0.75 (standard deviation (sd) 1.20), and of total body BMD -0.95 (sd 1.22), both significantly lower than reference values ($p<0.001$). Four patients (7 %), of whom one had not been treated with corticosteroids, had lumbar spine BMD SDS below -2 and eight patients (15 %), of whom three had not used corticosteroids before, had total body BMD SDS below -2. The BMD results are shown in Figure 1. BMD SDS results were similar in

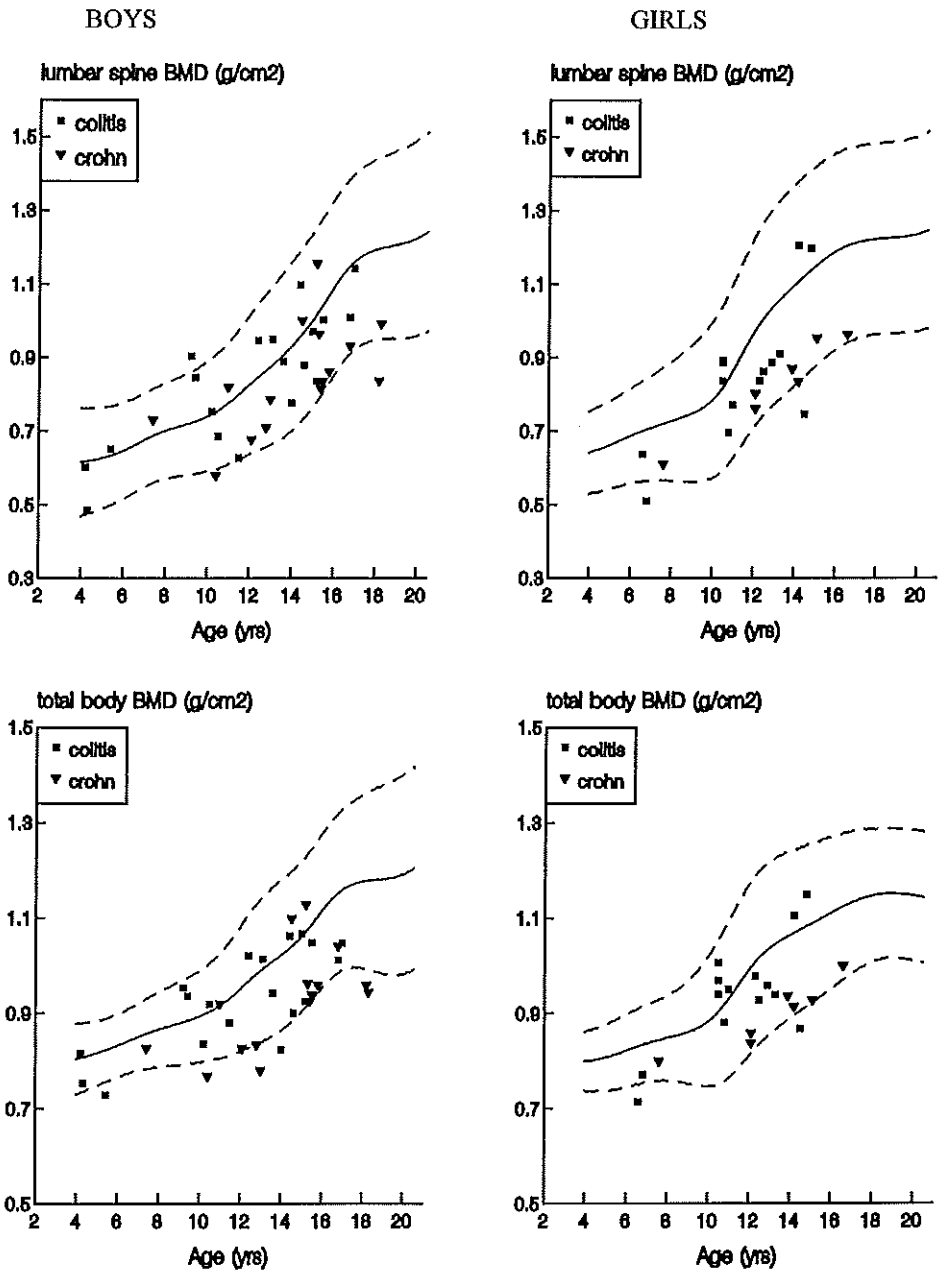


**Figure 1**

*Lumbar spine and total body bone mineral density results of the first measurement of 55 patients with inflammatory bowel disease. The continuous line represents the mean of reference values, the dotted lines +2 and -2 standard deviations.*

boys and girls.

The mean delay in bone maturation (chronological age minus bone age) was 0.71 year (sd 1.63). If the BMD SDS was calculated for bone age instead of chronological age, the mean lumbar spine BMD SDS was -0.47 (sd 1.04) and total body BMD SDS -0.80 (sd 1.04), both still significantly below zero ($p<0.01$ respectively $p<0.001$). The delay in bone maturation had a significant negative correlation with lumbar spine BMD SDS ($r=-0.47$, $p<0.001$) and total body BMD SDS ($r=-0.45$, $p<0.001$). The mean delay of puberty was 0.70 year (sd 1.14). A greater delay related to lower lumbar spine BMD SDS ($r=-0.45$, $p<0.01$) and total body BMD SDS ($r=-0.54$, $p<0.001$).

The mean height SDS was -0.54 (sd 1.25) and body mass index SDS -0.66 (sd 0.85), both significantly below zero ($p<0.01$ resp. $p<0.001$). Correlations of various variables with BMD SDS are listed in Table 1.

**Table 1**

*Correlation coefficients of various variables with lumbar spine and total body bone mineral density standard deviation score (BMD SDS) in 55 children with inflammatory bowel disease.*

| | lumbar spine BMD SDS | total body BMD SDS |
|---|---|---|
| height SDS | 0.54 *[1] | 0.59 *[1] |
| body mass index SDS | 0.19 | 0.36 *[3] |
| cumulative dose of prednisone (mg) | -0.32 *[2] | 0.19 |
| lean tissue mass SDS | 0.56 *[1] | 0.61 *[1] |
| fat mass SDS | 0.08 | 0.31 *[4] |

*[1] $p<0.001$, *[2] $p<0.01$, *[3] $p<0.02$, *[4] $p<0.05$

The cumulative dose of prednisone had a significant negative association with lumbar spine BMD SDS and not with total body BMD SDS.

The children with Crohn's disease had significantly lower lumbar spine and total body BMD SDS and height SDS than children with ulcerative colitis ($p=0.01$ for lumbar spine and $p=0.003$ for total body and height), even after adjustment for cumulative dose of prednisone.

The mean physical activity was 8.3 hours per week (sd 4.6) for boys and 5.9 hours per week (sd 3.1) for girls, both not significantly different from controls (respectively 9.2 and 7.1 hours per week). We found no correlation between physical activity and lumbar spine or total body BMD SDS.
Lumbar spine or total body BMD SDS did not differ between patients who had a previous ileocecal resection and patients who had no resection.
The duration of the disease did not relate to lumbar spine or total body BMD SDS.
In multiple regression analysis with diagnosis (Crohn's disease/ulcerative colitis), cumulative dose of prednisone, and body mass index SDS as determinants and BMD SDS as dependent variable, cumulative dose of prednisone and diagnosis related significantly to lumbar spine BMD SDS and explained 20 % of the variance. Only diagnosis related significantly to total body BMD SDS in this regression model ($r^2$ = 15 %).

*Body composition, caloric and calcium intake*
The mean SDS of lean tissue mass was -1.04 (sd 1.41), of bone mineral content -1.05 (sd 1.27), of fat mass -0.64 (sd 1.02), and of percentage body fat -0.38 (sd 1.11), all significantly lower than normal (all $p<0.001$, except for percentage body fat $p<0.02$). Correlations of these variables with BMD SDS are shown in Table 1. The SDS of percentage body fat had no significant correlation with BMD SDS. Cumulative dose of prednisone did not relate to lean body mass SDS or fat mass SDS. Patients with Crohn's disease had significantly lower lean body mass SDS ($p=0.003$) than patients with ulcerative colitis. They did not differ in fat mass or percentage fat SDS.
Twenty-six of the 36 children (73 %), whose food intake was analyzed, had a caloric intake below the recommended intake and 5 (14 %) had a low calcium intake.

*Biochemical parameters of bone metabolism*
Five children had a decreased 25 hydroxyvitamin D and one child a low 1,25 dihydroxyvitamin D. The 25 hydroxy- and 1,25 dihydroxy-vitamin D levels did not differ between patients with Crohn's disease and patients with ulcerative colitis. Osteocalcin was decreased in 3 of 40 patients (8 %) and IGF-I in 11 of 50 patients (22 %). All patients had normal PICP values. The patients with a low osteocalcin or a low IGF-I did not differ significantly in lumbar spine or total body BMD SDS, height SDS and body mass index SDS from the other patients. The hydroxyproline/creatinine ratio was low in 18 of 47 children (38 %), normal in 26 patients (55 %) and high in 3 patients (6 %). There was a significant difference in lumbar spine and total body BMD SDS between the patients with low, normal and high hydroxyproline/creatinine ratio (both $p<0.05$). If the ratio was higher the BMD SDS was lower. The other biochemical

results were within normal limits.

*Longitudinal data*

There was no significant change in BMD SDS between measurement one and two ($\Delta$ $SDS^1$). The mean $\Delta$ $SDS^1$ of lumbar spine BMD between the first two measurements was -0.07 (sd 0.48) and of total body BMD 0.06 (sd 0.51). The $\Delta$ $SDS^1$ of lumbar spine and total body BMD had no significant correlation with the cumulative dose of prednisone between the measurements.

Between measurement two and three there was a significant increase in BMD SDS ($\Delta$ $SDS^2$). The mean $\Delta$ $SDS^2$ of lumbar spine BMD was 0.46 (sd 0.73), and of total body BMD 0.61 (sd 0.48), both significantly higher than zero ($p<0.02$ for lumbar spine and $p<0.001$ for total body). The $\Delta$ $SDS^2$ of lumbar spine and total body BMD had a significant negative correlation with the cumulative dose of prednisone between measurement two and three ($r=-0.60$ for lumbar spine and $r=-0.56$ for total body, both $p<0.01$).

The increase in lumbar spine or total body BMD SDS of patients who were treated with calcium and vitamin D (n=12 between measurement one and two, n=6 between measurement two and three) did not differ from the increase in BMD SDS of the other patients. There was no significant change in SDS of the different body composition compartments, height SDS, body mass index SDS, and delay in bone maturation between measurement one and two and between measurement two and three.

*Comparison DXA and BIA*

There was a high correlation between lean tissue mass measured by DXA and lean body mass measured by BIA (r=0.98) (Figure 2A). The mean difference between DXA lean tissue mass and BIA lean body mass was 0.94 kg. The limits of agreements (-2 sd to +2 sd of the difference) were -4.0 to 5.9 kg (Figure 2B). If DXA lean body mass was calculated as lean tissue mass plus bone mass the mean difference with BIA lean body mass was higher (2.8 kg, limits of agreement -3.9 to 8.0 kg).

## Discussion

Bone mineralization was reduced in children and adolescents with IBD. BMD of lumbar spine as well as of total body were significantly lower than reference values. Both trabecular bone, present mainly in lumbar spine, as cortical bone, 80 % of the total skeleton[22], were affected.

Putative pathogenetic factors of osteopenia have been reported as the inflammatory

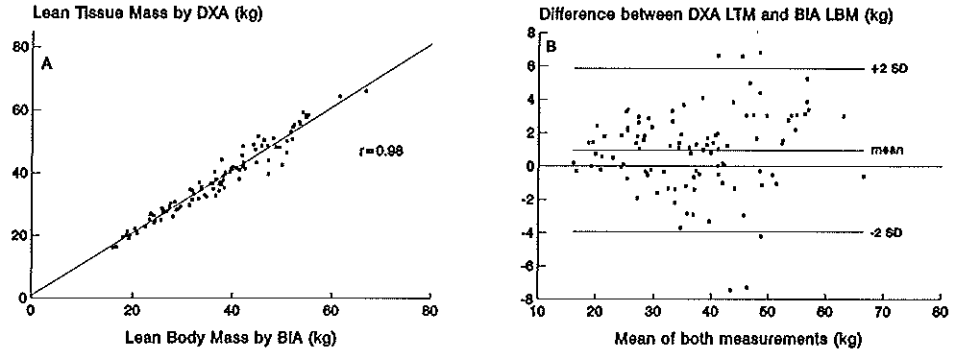


**Figure 2**

*A. Relation between lean tissue mass measured by dual-energy x-ray absorptiometry (LTM by DXA) and lean body mass measured by bio-electrical impedance analysis (LBM by BIA). The continuous line represents the regression line.*

*B. Difference between LTM by DXA and LBM by BIA plotted against the mean for the two methods.*

activity[23], steroid therapy[1], malnutrition[1], malabsorption[24], reduced calcium intake and vitamin D deficiency.[25]

Children with Crohn's disease had lower values of lumbar spine and total body BMD than children with ulcerative colitis. This is in agreement with Compston *et al.*[1] who reported that 41 % of the adult patients with Crohn's disease had decreased bone mass in contrast to only 14 % of patients with ulcerative colitis. At diagnosis a low BMD of both cortical bone as trabecular bone was found in adult patients with Crohn's disease but not in patients with ulcerative colitis.[24] Also growth failure in children has been reported to be more severe in children with Crohn's disease than in children with ulcerative colitis.[26, 27] The difference may be explained by the fact that Crohn's disease is a systemic illness while ulcerative colitis is a mucosal disorder.[24] The mechanism of osteopenia caused by the inflammatory process is unclear. Various cytokine abnormalities have been described in Crohn's disease[25], which may stimulate bone resorption.[29,30] Our results showed a negative relation between the bone resorption marker hydroxyproline and BMD and no relation between bone formation markers and BMD. An increase in bone resorption might account for a decrease in BMD in these patients.

Steroid therapy had a significant negative correlation with lumbar spine BMD and in the longitudinal data with the change in BMD of lumbar spine as well as of total body. Some studies reported a relation between corticosteroid and BMD in patients with chronic inflammatory bowel disease[1,31,32] and others not.[4] Corticosteroids are known to

have a negative influence on BMD, especially of trabecular bone.[33] Corticosteroids inhibit the inflammatory process in the intestine. Since the inflammatory process itself and the malabsorption as a consequence of the inflammation may also negatively influence BMD, the effect of corticosteroids on BMD is complex in patients with IBD. Besides, corticosteroids are used more often in patients with small bowel disease and in more severe types of inflammation.

IBD is characterized by a pattern of exacerbations of disease activity of variable severity interspersed with remissions. The variability of the disease might be the reason that we did not find a relation between the duration of the disease and BMD.

Body mass index, lean tissue mass and fat mass were decreased which may be related to the fact that a high proportion of the patients had an insufficient caloric intake. A low caloric intake has been reported before in patients with IBD.[27] Twenty-two percent of the children of the present study had a low IGF-I, which may be caused by undernutrition.[34] A higher body mass index, a parameter of the nutritional status, related to a higher BMD, which is also found in adults.[1,4] Nutritional recommendations have been shown to reverse growth impairment.[27]and will be beneficial for bone mineralization. Lean tissue mass SDS correlated positively with lumbar spine and total body BMD SDS. Increased lean tissue mass, and thereby muscle mass, may be related to more physical activity which may increase BMD.

Vitamin D deficiency may play a role in the pathogenetic process of bone loss. Subnormal serum 25 hydroxyvitamin D levels have been reported in patients with chronic IBD.[25,35] Ten percent of the patients of the present study had decreased vitamin D levels. In a study of 150 adult patients with inflammatory bowel disease no association was found between low BMD and low serum levels of 25 hydroxyvitamin D or elevated parathyroid hormone levels.[25] However, vitamin D might be of benefit in the treatment of osteopenia in these patients especially during corticosteroid therapy. Sambrook *et al.*[36] showed that treatment with calcium and calcitriol could prevent corticosteroid-induced bone loss of the lumbar spine. In the present study a selected group was treated with calcium and vitamin D and the change in BMD did not differ from the other patients. However, the aim of the study was not to investigate the effect of calcium and vitamin D therapy on BMD. Placebo-controlled studies in children with IBD are needed to evaluate the effect of treatment with calcium and vitamin D on BMD in these patients.

Delay in skeletal maturation and puberty had a significant negative correlation with BMD. However, the decrease in BMD could not be explained by the retardation in bone maturation. BMD SDS calculated for bone age was also reduced. The delay in BMD might catch up at a later age. The catch up of BMD may not be complete showed by a study of BMD of men with late puberty.[37] Adult men who had had late

puberty had a lower BMD than men who had had a normal timing of puberty.
The correlation between lean tissue mass measured by DXA and lean body mass measured by BIA was high and the difference small. The difference of BIA lean body mass and DXA lean tissue mass was smaller than the difference between BIA lean body mass and DXA lean body mass, which includes bone mass. It seems as BIA lean body mass excludes bone mass. If BIA fat mass is calculated as weight minus BIA lean body mass, BIA fat mass is overestimated if DXA is taken as the standard. In a previous study DXA showed to be an accurate method of measuring soft tissue composition by cross-calibration with chemical analysis after postmortem homogenization.[38,39] However, the advantage of BIA is that it is cheap, quick and uses portable equipment. Our results showed that BIA is a reliable method to estimate lean tissue mass in comparison with DXA.
From this study, it can be concluded that the mean bone mineral density of children with IBD was lower than reference values. Osteopenia appears to be more common in children with Crohn's disease than in children with ulcerative colitis. Corticosteroid therapy and nutritional status are determinants of BMD in these patients. Long-term longitudinal studies are needed to investigate whether these children will attain a normal peak bone mass or may benefit from calcium and vitamin D supplement.

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# Chapter 5

# Bone mineral density and bone metabolism of prepubertal children with asthma after long-term treatment with inhaled corticosteroids

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## Abstract

Little is known about the effect of long-term treatment of asthmatic children with inhaled corticosteroids (ICS) on bone mineral density (BMD). In the present cross-sectional study BMD, bone metabolism, height, body composition and bone age were evaluated in 40 prepubertal children (21 boys) with asthma, treated with a moderate to high dose of ICS during 3 to 8 years. Body composition, BMD of lumbar spine and total body were measured by Dual Energy X-ray Absorptiometry. BMD results were compared with 148 prepubertal healthy children of the same population. Blood samples were taken for the determination of biochemical bone parameters. The asthmatic children had a decreased height, lean tissue mass and fat mass and a delay of bone maturation, indicating growth retardation. Asthma on ICS had a significant negative relation with total body BMD in a multiple regression model with adjustment for age, sex, height and weight ($p=0.01$). Duration of ICS use correlated negatively with total body BMD if it was added to the model ($p=0.01$). Asthma on ICS had no significant relation with lumbar spine BMD. If age of the asthmatic children was replaced by their bone age in the model no significant correlation was found between asthma on ICS and total body or lumbar spine BMD. The biochemical parameters of bone metabolism were within normal limits. In conclusion, children with asthma who had used ICS daily for 3 to 8 years had lower total body BMD than healthy controls. Long-term longitudinal studies are needed to investigate if these children attain a normal peak bone mass.

## Introduction

Inhaled corticosteroids (ICS) are commonly prescribed for moderate to severe asthma. Whether ICS have systemic effects on growth and bone metabolism remains controversial. In studies with healthy adult subjects a decrease of osteocalcin, a parameter of bone formation, was found during ICS use.[1-4] Adults with asthma treated with high dose of ICS had lower bone mineral density (BMD) than patients who did not use ICS[5] or healthy controls.[6] Studies of BMD in asthmatic children receiving ICS reported no lower BMD in patients than controls.[7-11] In these studies the treatment period was 6 months up to 2 years. Wolthers *et al.*[12] evaluated the short term linear growth of asthmatic children by knemometry and found a dose related suppression during treatment with ICS. Prolonged administration of 200 μg of ICS daily to young children with asthma did not impair growth or pituitary-adrenal function[13], but growth velocity decreased with higher doses.[14]

The aim of the present study was to evaluate the effect of long-term treatment with moderate to high dose ICS on BMD and bone metabolism in children with asthma. For this purpose, we performed a cross-sectional study of prepubertal asthmatic children who received continuous daily treatment with ICS during more than three years, and compared these to healthy controls with respect to the BMD of the lumbar spine and total body, height, body mass index, body composition and bone metabolism.

## Materials and methods

*Patients and controls*

Forty prepubertal children (21 boys and 19 girls) with asthma, who had used ICS continuously for at least three years in a prescribed dose of at least 0.4 milligram per day, were included in the study. Age ranged from 4 to 11 years (median 7). Duration of ICS use ranged from 3.0 to 8.2 years (median 4.1) and lifetime cumulative dose of ICS from 529 to 2719 mg (median 969). Budesonide was used by 25 children, beclomethasone dipropionate by 15 children. They had not received oral corticosteroids during the previous half year. The number of short oral prednisolone courses patients had received during their life ranged from 0 to 18 (median 4). Three boys and 5 girls had never received systemic corticosteroids. Median duration of continuous treatment with inhaled $beta_2$-agonists was 3.3 years (range 0.2 to 6.5). Twenty-nine patients had used cromoglycate and 16 patients had used theophylline. Nearly all children had used one or several courses of antibiotics.

Controls for BMD were selected from the same population and had a similar age distribution as patients (median age was 7 years). They were 148 prepubertal healthy children, 79 boys and 69 girls, who had participated in a study of BMD normal values, published before.[15]

The biochemical bone parameters osteocalcin, the carboxy terminal propeptide of type I collagen (PICP), the cross-linked telopeptide of type I collagen (ICTP) were compared with local reference values (based on 25, 82, and 88 samples respectively). These controls were prepubertal children with mild diseases which are known not to affect bone metabolism, who attended the out-patient clinic and from patients undergoing minor surgery before anaesthesia. Informed consent was obtained from parents of all participating children.

*Measurements*

Height was assessed using a fixed stadiometer. Weight was measured without shoes on a calibrated standard clinical balance. Body mass index was calculated as weight

divided by square height ($kg/m^2$). Height and body mass index of patients was expressed as standard deviation scores (SDS) as compared to Dutch reference values[16,17] to assess possible growth retardation. Daily calcium intake was estimated through a food-frequency questionnaire of dairy products.[18] BMD of the lumbar spine and total body was measured by Dual Energy X-ray Absorptiometry (Lunar, DPXL/PED, Lunar Radiation Corporation, Madison, Wisconson, USA). This equipment also gives estimates of body composition as lean tissue mass, fat mass and bone mineral content. Total tissue mass is the sum of these variables. Percentage body fat is calculated for total tissue mass. Lean tissue mass, fat mass and percentage body fat were compared to our age- and sex-matched Dutch reference values[19] and expressed as SDS.

In 37 patients an X-ray of the left hand was made to assess bone age (parents of 3 patients refused this X-ray). Skeletal maturation was determined by one investigator (AB) according to the Tanner-Whitehouse Radius-Ulna-Short bones (RUS) method.[20]

Non-fasting morning blood samples were obtained of 29 patients (parents of 11 patients refused venapuncture) to evaluate bone metabolism. In these samples measurements were performed of calcium, phosphate, alkaline phosphatase, parathyroid hormone, osteocalcin, PICP, ICTP, insulin-like growth factor 1 (IGF-I) and insulin-like growth factor binding protein 3 (IGFBP-3). Urine (first morning void) of 23 patients was examined for calcium, hydroxyproline and creatinine. Ratio's of calcium and creatinine (CA/CR) and of hydroxyproline and creatinine (OHPR/CR) were calculated. Osteocalcin, intact PTH (1-84), PICP, and ICTP levels were measured by radioimmunoassay (Incstar Corporation, Stillwater, USA, and Orion Diagnostica, Espoo, Finland). For assessment of IGF-1 and IGFBP-3 radioimmunoassay kits of Med-Genix Diagnostics, Fleurus, Belgium and Diagnostic System Laboratories, Webster, Texas were used. IGF-I and IGFBP-3 age- and sex-specific reference values were based on 600 samples of healthy Dutch children.[21]

*Statistics*

Possible growth retardation among patients was evaluated with height, body mass index, fat and lean tissue mass SDS, which were age- and sex standardised. A possible deviation from the expected zero was tested with one sample t-tests.

BMD values of patients were compared with those of controls by linear multiple regression analysis, adjusting for age, sex, height and weight. This method provides the effect of asthma on BMD, independent of possible growth retardation. Additionally, the influence of ICS therapy was assessed by adding duration of therapy or cumulative dosage of ICS as linear variable in the mentioned regression model. In these models controls were given zero ICS values. These models enabled us to study the additive effect of ICS therapy in asthma on BMD.

The difference of means of two groups of variables with a normal distribution were tested with a two-sample t-test. A two-sided p-value $< 0.05$ was considered significant.

## Results

Table 1 shows the results of height, body mass index and body composition variables SDS of the asthmatic children. The mean delay in bone maturation (chronological age minus bone age) was 0.9 year (sd 1.3). Height, lean tissue mass and fat mass SDS were significantly smaller than normal. Therefore, adjustment for body size was performed in the evaluation of BMD.

**Table 1**
*Mean standard deviation scores (SDS) and range of several variables in 40 asthmatic children.*

| | mean SDS | range |
|---|---|---|
| total body : | | |
| lean tissue mass | -0.92[a] | -2.30 to 0.60 |
| fat mass | -0.34[b] | -1.92 to 2.26 |
| percentage body fat | -0.09 | -2.07 to 2.79 |
| height | -0.52[c] | -2.66 to 1.81 |
| body mass index | 0.28 | -1.62 to 3.65 |

[a] $p<0.001$, [b] $p<0.05$, [c] $p<0.01$, significantly smaller than zero.

In the multiple regression model with adjustment for age, sex, height and weight, asthma had a significant negative correlation with total body BMD ($p=0.01$). The analysis resulted in the following model : total body BMD ($g/cm^2$) = 0.8166 - 0.005883 x sex (0=male, 1=female) + 0.005025 x weight (kg) - 0.001422 x height (cm) + 0.01083 x age (yrs) - 0.02009 x asthma (0=no asthma, 1=asthma on ICS). The values of total body BMD of the patients are shown in Figure 1. There was no significant difference in BMD between boys and girls. In this model asthma had no significant relation with lumbar spine BMD.

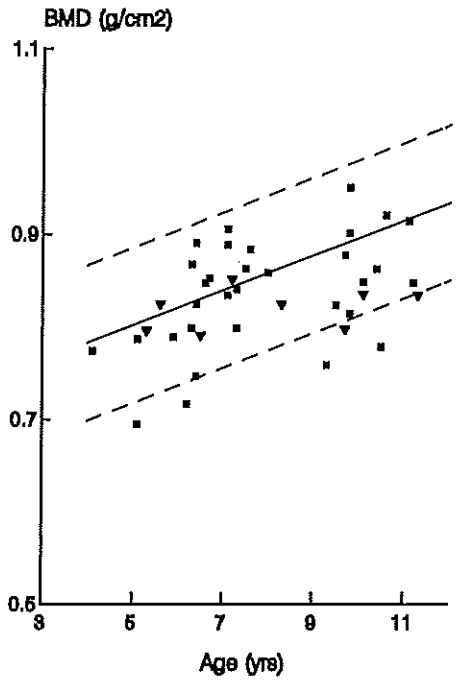


**Figure 1**
*Total body bone mineral density (BMD) of 40 asthmatic children. The line represents the regression line of the controls; the dotted lines the 95 % prediction intervals. The triangles represent the results of patients who had never received systemic corticosteroids.*

The duration of ICS use had a significant negative correlation with total body BMD if it was added to the above described model (p=0.01). No relation with lumbar spine BMD was found.
The cumulative dose of ICS, the number of oral corticosteroid courses during lifetime, drugtype (either budesonide or beclomethasone), and calcium intake did not relate to lumbar spine or total body BMD in the multiple regression model. The mean calcium intake (1159 mg/day) did not differ significantly from the controls (1240 mg/day).
If age of the asthmatic children was replaced by their bone age in the above described model no significant correlation was found between asthma and total body or lumbar spine BMD.

*Biochemical parameters of bone metabolism*
All asthmatic children had osteocalcin, PICP and ICTP values within the normal limits (± 2 SD). The mean values and 95 % central range are shown in Figure 2. The mean values did not differ significantly from the mean of the reference values. All patients except one had normal IGF-I and IGFBP-3 values. Calcium, phosphate, alkaline phosphatase, parathyroid hormone were all within normal limits.

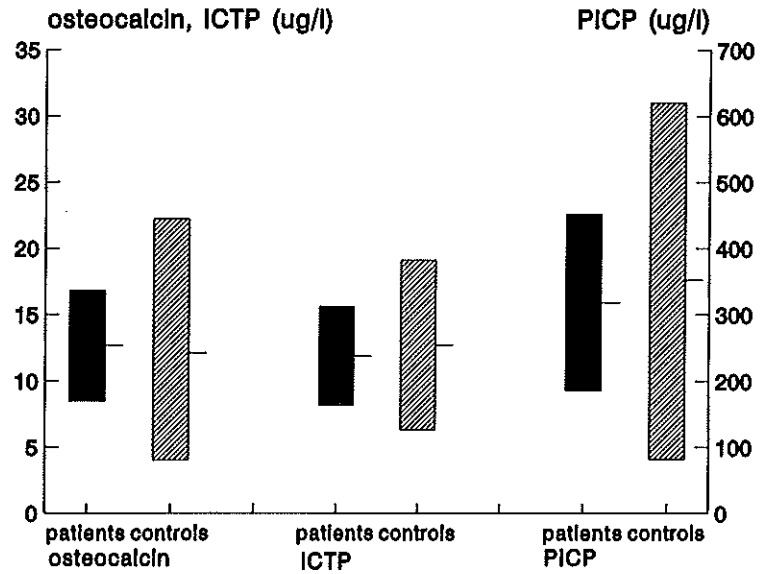


**Figure 2**

*Results (mean and 95 % central range) of osteo-calcin, carboxy terminal propeptide of type I collagen (PICP), cross-linked telo-peptide of type I collagen (ICTP) of the asthmatic patients compared to reference values.*

## Discussion

Asthma and duration of ICS use had a significant negative relation with total body BMD in the analysis of 40 prepubertal asthmatic children and 148 healthy controls.
Previous studies of BMD in children with asthma using ICS did not find a lower BMD compared to controls.[7-11] Differences with the present study are the shorter duration of ICS use[7-11] and lower ICS dosage.[7, 9-11] Some studies evaluated lumbar spine and not total body BMD[9,10] or included both prepubertal and pubertal children.[7-9,11] The patients of the present study were prepubertal, used ICS continuously for a long time in a moderate to high dose. The control group for BMD were children who were not selected on being non-asthmatic. In the general population approximately 10 % has mild asthma. Maybe the difference in BMD between the asthmatic children and the controls would have been greater had the asthmatic children been compared with verified non-asthmatics.
The decreased BMD in these children may be explained by ICS use but also by

previous use of oral prednisolone and/or the chronic illness itself and its consequences, including malnutrition and inactivity. Total body BMD had a negative correlation with the duration of ICS treatment, which suggests a causal dose-effect relation. However, the cumulative dose of ICS had no significant relation with total body BMD. The period of ICS use is a marker of duration and severity of asthma. Therefore the reduction of total body BMD may be due to the chronic illness rather than to the effect of ICS use. This is supported by finding a decrease in total body BMD, which consists of 80 % cortical bone[22], and not in lumbar spine BMD, which contains a higher proportion trabecular bone. Corticosteroids influence mainly trabecular bone.[23,24] For the same reason it is improbable that the decrease in BMD is caused by previous courses of systemic corticosteroids. Besides, the patients who had never used systemic corticosteroids did not differ in BMD from the other asthmatic children.

The asthmatic patients had growth retardation shown by a decreased height SDS and delay in bone maturation. A decrease in BMD and growth retardation may be caused by malnutrition. We found a decreased lean body mass and fat mass SDS. However, the percentage body fat and body mass index SDS, which are adjusted for body size, were normal. The levels of IGF-I were normal, whereas we would expect low IGF-I levels in case of malnutrition.[25] Therefore, we think that malnutrition does not explain the decreased BMD and growth retardation. Crowley *et al.*[26] found decreased growth rate in children with asthma and ICS use. The effect was not reflected in changes in the secretion of GH or IGF-I and normal weight and skinfolds thickness did not indicate inadequate nutrition.[26]

Children with a chronic illness may have a decreased physical activity. This may have a negative influence on BMD. We did not estimate physical activity in the present study.

Most patients had a delay in bone maturation. BMD for bone age was not reduced. It may be that the decrease in BMD will be caught up at a later age. Studies concerning growth showed that many asthmatic children had a delay in skeletal maturation and delayed onset of puberty which did not affect final height.[27,28] There are no studies concerning peak bone mass in asthmatic children. A study of Finkelstein *et al.*[29] found that young males who had had late puberty had lower BMD than men who had a normal timing of puberty. BMD did not catch up completely in these subjects. BMD increases with age till the peak bone mass is reached between the age of 20 and 30 years.[30] Peak bone mass is a major determinant of osteoporosis later in life.[30,31] Longitudinal studies are needed to know if BMD of asthmatic children with long-term use of a moderate to high dose of ICS catches up and if a normal peak bone mass is attained.

Several studies showed that ICS might have a deleterious effect on bone metabolism.

Osteocalcin level decreased in healthy subjects during ICS use.[1-4] Asthmatic patients with ICS use had lower osteocalcin levels than asthmatics without ICS use.[32,33] Birkebaek *et al.*[34] showed that short-term treatment with a high dose ICS (800 μg per day) suppressed PICP in asthmatic children. Osteocalcin is generally presented as a marker of bone formation. It is synthesised by osteoblasts and incorporated in bone matrix. Some osteocalcin leaks into the circulation.[35] PICP is a more specific indicator of bone formation than osteocalcin.[36] It is liberated at the deposition of type I collagen, the major component of bone matrix. We also evaluated ICTP, a marker of bone resorption. ICTP is released during bone resorption by the degradation of collagen type I.[35] Both bone formation and bone resorption markers were within normal limits, indicating that there is a normal balance between bone formation and bone resorption in these asthmatic children. This agrees with what was found in a study of long-term effects of ICS on bone metabolism in adults with airway obstruction.[37] During two and a half years of treatment with ICS no change in ICTP and PICP was found.

In conclusion, children with asthma who had used a moderate to high dose of ICS for 3 to 8 years had a decreased total body BMD. Long-term longitudinal studies of asthmatic children are needed to evaluate whether these findings reflect a transient decrease in BMD or a continuous deviation from normal.

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# Chapter 6

# Bone mineral density in children with acute lymphoblastic leukaemia

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## Abstract

Lumbar spine and total body bone mineral density (BMD) and bone metabolism were evaluated in children with acute lymphoblastic leukaemia (ALL) at diagnosis, during treatment with chemotherapy and one year after completion of treatment. Thirty-two children (21 boys and 11 girls) participated in the study. Fourteen children started the study at diagnosis and 18 during or after the treatment period. Lumbar spine and total body BMD were measured with dual energy x-ray absorptiometry, and expressed as standard deviation scores (SDS). Blood samples were obtained to assess bone metabolism.

Three of 14 children had low lumbar spine BMD at diagnosis. All children had normal total body BMD. Markers of bone turnover were depressed. Total body BMD SDS decreased significantly during the first year of treatment ($p<0.001$). Lumbar spine BMD SDS did not change significantly. Parameters of bone turnover increased to normal during the treatment period. Parathyroid hormone had increased significantly after 1 year ($p<0.05$). Mineral homeostasis was disturbed in some patients during treatment. Three of 7 patients had low total body BMD 1 year after completion of treatment. All patients had normal lumbar spine BMD and normal biochemical results at that time.

In conclusion, lumbar spine BMD and bone turnover were decreased in some patients at diagnosis. Total body BMD decreased significantly during treatment and was low in 3 of the 7 patients one year after completion of the treatment.

## Introduction

Skeletal abnormalities and bone pain are known to occur in childhood acute lymphoblastic leukaemia (ALL).[1] Fractures and osteoporosis have been described in association with ALL.[2-5] One study showed that at diagnosis 13 percent of 40 children with ALL showed radiographic osteopenia.[2] One case report described osteopenia and vertebral fractures after induction treatment in an adolescent patient with ALL.[3] Disturbances in mineral metabolism and low bone mass of the radius, measured with single photon absorptiometry, were reported during therapy in children with ALL.[4] Survivors of childhood ALL who had received cranial irradiation had osteopenia while nonirradiated survivors had normal bone mass.[5] Nussey *et al.* showed that adult survivors of childhood ALL with untreated growth hormone deficiency had reduced bone mineral density while patients without growth hormone deficiency or patients who received growth hormone treatment had normal bone mineral density.[6] The objective of

the present study was to evaluate bone mineral density (BMD) and bone metabolism of children with ALL at diagnosis, during treatment with chemotherapy and without cranial irradiation, and one year after cessation of treatment.

## Patients and Methods

Thirty-two children (21 boys and 11 girls) participated in the study. Between June 1994 and November 1995 all 14 newly diagnosed patients with ALL older than 4 years were enroled consecutively in the study. Twenty-two patients under treatment or 1 year after cessation of treatment for ALL without a relapse were asked to participate in the study of whom 4 refused. The mean age of the patients at the time they started the study was 7.9 years (standard deviation (sd) 4.0). All children were treated according to protocol ALL-8 of the Dutch Childhood Leukaemia Study Group. None of them received cranial irradiation. This protocol resembles the Berlin-Frankfurt-Münster (BFM)-ALL 90 study.[7] Nine patients were in the standard, 19 in the medium and 4 in the high risk group.[7] Chemotherapy involved vincristine, prednisone or dexamethasone, daunorubicine, L-asparaginase, methotrexate, cytosine-arabinoside, cyclofosfamide, 6-mercaptopurine, adriamycine, 6-thioguanine and in the high risk group additional vindesine, ifosfamide and etoposide. Maintenance therapy included low dose methotrexate, 6-mercaptopurine and in some patients L-asparaginase. Treatment is completed in 2 years. Patients were excluded from further study in case of a relapse (n=3).

The points of time of measurements were at diagnosis ($t_0$), after treatment for 6 months ($t_{½}$), 1 year ($t_1$) and 2 years ($t_2$), and 1 year after cessation of treatment ($t_3$).

BMD of 4 children was measured at $t_0$, $t_{½}$, $t_1$ and $t_2$; of 1 child at $t_0$, $t_1$ and $t_2$; of 4 children at $t_0$, $t_{½}$ and $t_1$; of 1 child at $t_0$ and $t_{½}$ and of 4 children at $t_0$ and $t_1$. Eighteen other children started during and after treatment : two were measured at $t_{½}$ and $t_1$, one at $t_1$ only, one at $t_1$ and $t_2$, one at $t_1$, $t_2$ and $t_3$, seven at $t_2$ only, and six at $t_3$ only.

BMD (g/cm$^2$) of lumbar spine and total body was measured by Dual Energy X-ray Absorptiometry (DXA, Lunar DPXL/PED, Madison, Wisconsin, USA). The results were compared to healthy age- and sex-matched controls of the same population and expressed as standard deviation scores (SDS).[8] DXA total body measurement also gives estimates of body composition as lean tissue mass (g), fat mass (g) and bone mineral content (g). Lean tissue mass, fat mass and percentage of body fat were compared to our age- and sex-matched Dutch reference values and expressed as SDS.[9]

Height was assessed with a fixed stadiometer, compared to Dutch reference values and expressed as SDS.[10]

Blood samples were obtained at the same time as BMD measurements. In these samples assessments were performed of calcium, phosphate, parathyroid hormone (PTH), osteocalcin and procollagen type I C-terminal propeptide (PICP), carboxy-terminal telopeptide of type I collagen (ICTP), insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein 3 (IGFBP-3). Osteocalcin, intact PTH were measured by radioimmunoassay (Incstar Corporation, Stillwater, USA). For measurement of PICP and ICTP radioimmunoassay kits of Orion Diagnostica, Espoo, Finland were used with coefficients of variation of 4-6 % and 4-8 % respectively. For prepubertal children our own reference values of osteocalcin, PICP and ICTP were used, based on respectively 25, 82 and 88 samples. Reference values for the older children were taken from other studies which used the same assays.[11-13] Assessment of IGF-1 and IGFBP-3 was performed with radioimmunoassay kits of Med-Genix Diagnostics, Fleurus, Belgium and Diagnostic System Laboratories, Webster, Texas. IGF-I and IGFBP-3 age- and sex-specific reference values were based on 600 samples of healthy Dutch children.[14] Samples for assessment of osteocalcin, PICP, ICTP, vitamin D and PTH missed in four patients and of IGF-I and IGFBP-3 in three patients at diagnosis. Samples for these assessments missed at $t_{½}$ in two patients, at $t_1$ in one patient and at $t_3$ in four patients.

*Statistics*

Comparison of the mean SDS from the expected zero was tested with one sample t-test. The within patient change was tested if it differed from zero with one sample t-test. Two groups of variables with a normal distribution were compared with a two-sample t-test. Oneway analysis of variance was used to compare the three risk groups.

## Results

At diagnosis the mean SDS of lumbar spine BMD was -0.67 (sd 1.3) and of total body BMD 0.02 (sd 1.3), both not significantly different from zero. However, 3 of the 14 children had lumbar spine BMD SDS below -2. None of the children had total body BMD SDS below -2. During the first 6 months lumbar spine BMD SDS remained stable, but total body BMD SDS decreased significantly (mean $\Delta$ -0.59, $p<0.001$) (Figure 1). The mean change in total body BMD SDS between $t_0$ and $t_1$ was -1.10 (sd 0.69), significantly lower than zero ($p<0.001$). Both mean BMD SDS of lumbar spine and total body at $t_{½}$ were significantly below zero ($p<0.02$ for lumbar spine and $p<0.05$

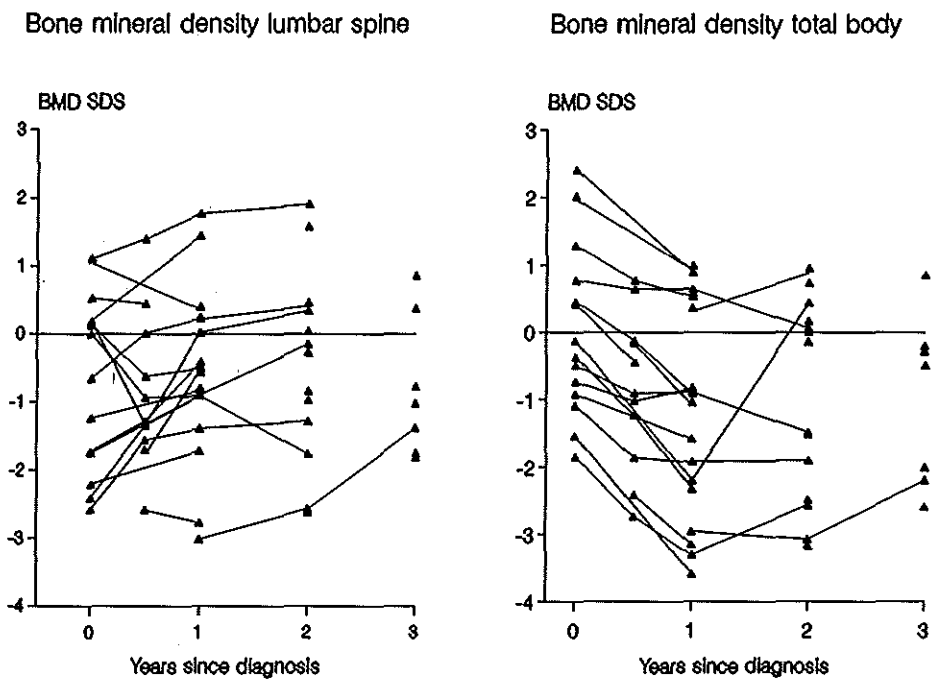


**Figure 1**

*Standard deviation scores (SDS) of lumbar spine and total body bone mineral density (BMD) before, during and 1 year after cessation of treatment in children with acute lymphoblastic leukaemia. The lines connect measurements within one patient.*

for total body). This was also the case for mean total body BMD SDS at $t_1$ ($p<0.01$) and $t_2$ ($p<0.05$).

At $t_3$ mean BMD SDS of lumbar spine and total body were respectively -0.78 (sd 1.04) and -0.99 (sd 1.27), both not significantly different from zero. None of the children had lumbar spine BMD SDS below -2, three children had total body BMD SDS below -2.

There was no difference in BMD SDS between the sexes. At diagnosis BMD SDS did not vary significantly between the standard-, medium- and high-risk group. The decrease in total body BMD SDS did not differ between the three risk groups.

At diagnosis, height SDS was not significantly different from zero; only one patient had height SDS below -2. Height SDS decreased during the first 6 months (mean $\Delta$ -0.33, $p<0.001$). From $t_{½}$ to $t_3$ height SDS did not change significantly. At $t_3$, two

patients had height SDS below -2.

At diagnosis, mean lean tissue mass SDS was significantly lower than zero (-0.74, $p<0.01$). Fat mass and percentage fat SDS did not differ significantly from zero. Lean tissue mass, fat mass and percentage fat SDS did not change significantly during treatment.

Five patients (16 %) had a fracture at diagnosis or during the treatment period. One girl with Down syndrome had multiple thoracal vertebral impression fractures at diagnosis. She started the study at $t_1$. At that time her lumbar spine and total body BMD SDS were both -3.0. Fractures of the humerus, os cuneiforme, os metatarsalis V and forearm occurred in the other 4 patients after traumas during treatment. One of them had both lumbar spine and total body BMD SDS below -2 and one had total body BMD SDS below -2.

At diagnosis mean osteocalcin and PICP, both parameters of bone formation, and ICTP, a marker of bone resorption, were significantly lower compared to controls ($p<0.001$ for osteocalcin and ICTP, $p<0.05$ for PICP). Osteocalcin was below the normal range (less than -2 sd) in 5, PICP in 1 and ICTP in none of the 10 children. All three biochemical parameters had increased significantly after 6 months of treatment ($p<0.01$ for osteocalcin and PICP, $p<0.001$ for ICTP). All children had normal values of osteocalcin, PICP and ICTP at $t_{½}$, $t_1$, $t_2$ and $t_3$ and the means did not differ significantly from the means of controls.

Six of the 14 patients had low alkaline phosphatase at diagnosis. After one year, alkaline phosphatase had increased significantly ($p<0.01$). During and after treatment none of the patients had low alkaline phosphatase.

At diagnosis, 2 of the 14 patients had hypocalcaemia, 2 had hyperphosphatemia, 2 had low PTH, 1 had high PTH, 1 had low 25 hydroxyvitamin D and 2 had low 1,25 dihydroxyvitamin D. During the two years of treatment, 7 of 25 patients had decreased serum calcium, 2 had increased phosphate, 2 had high PTH, 4 had low 25 hydroxyvitamin D and 2 had low 1,25 dihydroxyvitamin D in at least one measurement. Mean PTH, 1,25 dihydroxyvitamin D and calcium had increased significantly after 1 year ($p<0.05$ for PTH and 1,25 dihydroxyvitamin D, $p<0.02$ for calcium). Phosphate and 25 hydroxyvitamin D did not change significantly. At $t_3$, one year after treatment, all had normal serum calcium, phosphate, PTH and 1,25 dihydroxyvitamin D. Two patients had low 25 hydroxyvitamin D.

At diagnosis, 5 of 11 patients had low IGF-I. IGFBP-3 was normal in all patients. IGF-I and IGFBP-3 increased significantly during the first 6 months (both $p<0.01$). During the two years of treatment, 5 of 20 patients had low IGF-I in at least one measurement. At $t_3$, all had normal IGF-I and IGFBP-3.

At diagnosis, 1,25 dihydroxyvitamin D correlated positively with lumbar spine BMD

SDS ($p<0.05$), total body BMD SDS ($p<0.01$) and IGF-I levels ($p<0.02$). During and after treatment no correlation was seen. Other biochemical parameters had no correlation with lumbar spine or total body BMD SDS. The biochemical markers did not relate to the decrease of total body BMD.

## Discussion

At diagnosis, 3 of 14 (21 %) children with ALL had a low lumbar spine BMD. Markers of bone turnover were reduced. Total body BMD decreased during the first year of treatment, suggesting a negative effect of chemotherapy or other factors like decreased physical activity on cortical bone.

At diagnosis of ALL, biochemical markers indicated suppression of bone turnover by the disease. This is in agreement with findings of Halton *et al.*[2], who reported low osteocalcin and low urinary deoxypyridinoline, a parameter of bone resorption, in children with ALL at diagnosis. Biopsy specimens of iliac bone showed abnormalities in bone mineralization in three of nine children.[2] Leukaemic infiltration of the bone marrow and marrow expansion have been mentioned as pathogenic factors of osteoporosis in ALL.[3] This may have caused the low lumbar spine BMD and suppression of bone turnover found in some patients. Lumbar spine has a high content of trabecular bone, which contains a high proportion of bone marrow. Trabecular bone is metabolically more active than cortical bone.

Lumbar spine and total body BMD SDS seemed to be negatively affected by low levels of 1,25 dihydroxyvitamin D at diagnosis in agreement with findings of Halton *et al.*.[2] Reduced production of 1,25 dihydroxyvitamin D is related to low IGF-I levels.[15]

During treatment, markers of bone turnover increased to normal levels. However, hypocalcaemia, hyperparathyroidism and low 25 hydroxyvitamin D were demonstrated in some patients. Atkinson *et al.*[4] also showed alterations in calcium and vitamin D metabolism during treatment for ALL in children. About 50 % of the patients demonstrated hypocalcaemia and hypomagnesemia and 12 of the 13 patients had low 1,25 dihydroxyvitamin D levels.[2] The increase of PTH in the present study may have caused the decrease in total body BMD, which consists for about 80 % of cortical bone.[16] Hyperparathyroidism mainly affects cortical bone.[17] Vitamin D preparations suppress PTH secretion by both raising serum calcium and inhibiting PTH gene transcription.[18] Therapy with vitamin D might prevent or diminish the decrease of total body BMD and may prevent fractures of cortical bone.

Methotrexate and corticosteroids both can influence calcium metabolism. Osteoporotic fractures secondary to high doses of methotrexate in children with ALL were

described.[19] Besides, osteoporosis was reported in patients treated with low doses of methotrexate for psoriasis and rheumatoid arthritis.[20] In rats, low-dose methotrexate caused a decrease in trabecular bone mass and decreased osteoblast activity, while cortical bone mass was not affected.[21] It is unknown if high-dose methotrexate influences cortical bone. In the protocol of the present study high-dose methotrexate is given during the first months of treatment when the decrease in total body BMD is observed. During maintenance treatment low-dose of methotrexate is prescribed.

Corticosteroids mainly affect trabecular bone[22], present in the vertebrae. In the protocol of our study, corticosteroids are prescribed during the first half year of treatment. Since, in the present study total body BMD decreased and not lumbar spine BMD, corticosteroids may not be the causal factor. It is unknown if other cytotoxic drugs affect bone metabolism.

A decrease of physical activity during the treatment period could negatively influence BMD. Physical activity was not estimated in our study. Physical activity of the patient may decrease during treatment, however, lean tissue mass, mainly muscle mass, remained stable.

Gilsanz *et al.*[5] reported normal spine BMD in survivors of ALL who did not receive cranial irradiation a half year to 8 years after completion of treatment. This is in agreement with the present study. Normal BMD of the femoral neck, mainly cortical bone, was found in adult survivors of childhood ALL without growth hormone deficiency.[6]

In conclusion, lumbar spine BMD and bone turnover were decreased in some patients at diagnosis. Total body BMD decreased during the first year of treatment in children with ALL, which might be caused an increase of PTH during chemotherapy. Bone turnover increased to normal. One year after cessation of treatment three of 7 children still had a low total body BMD. Further studies are needed to evaluate the long-term effect on BMD in these patients and if a decrease of BMD can be prevented with vitamin D treatment.

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# Chapter 7

# Bone mineral density in renal diseases

# Chapter 7.1

# Renal transplantation and osteoporosis

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## Abstract

A cross-sectional study assessed the bone mineral density (BMD) of 20 young adult patients who received a renal transplantation in childhood. The BMD of the lumbar spine, mainly trabecular bone, and of the total body, mainly cortical bone, were measured and expressed as standard deviation score (SDS).

Fourteen patients (70%) had a BMD SDS of the lumbar spine below -1, of whom 6 patients below -2. Fifteen patients (75%) had a BMD SDS of the total body below -1, of whom 7 patients below -2. Both trabecular and cortical bone appeared to be involved in the osteopenic process.

The cumulative dose of prednisone was inversely correlated to both lumbar spine and total body BMD SDS. In a multiple regression analysis the cumulative dose of prednisone appeared to be the only factor with a significant effect on BMD SDS.

Most young adult patients who had received a renal transplantation in childhood had moderate to severe osteopenia. Corticosteroid treatment played a major part in the development of osteopenia in these patients.

## Introduction

Metabolic bone disease is a known complication in patients with chronic renal failure. Secondary hyperparathyroidism has a central role in the pathogenesis of renal osteodystrophy.[1] Although successful renal transplantation corrects most metabolic disturbances that cause renal osteodystrophy, osteopenia remains a problem.[2-5] Bone mass is influenced by renal osteodystrophy, corticosteroid therapy, delayed puberty, and diminished physical activity.[2]

Most studies evaluating bone mass of renal transplant recipients involved patients transplanted in adulthood.[2,3,5] These studies showed a decrease in bone mass after renal transplantation.

As adolescence is a crucial time for the development of bone mass[6,7] we evaluated the bone mineral density (BMD) of young adult patients who received their first renal transplantation before the age of 16 years. In addition, we studied the influence of various factors that could affect bone mass such as prednisone treatment, age and pubertal stage at first transplantation, delayed puberty, number of transplantations and total duration of dialysis.

## Patients and methods

*Patients*

Twenty recipients of renal transplants were studied (13 men and 7 women). These patients met the following criteria: first renal transplantation before the age of 16 years and present age between 20 and 30 years. The clinical data of the patients are summarized in Table 1. Mean chronological age at the time of the study was 25 years (range 21 to 29). Mean age at the first transplantation was 12.1 years (range 7.1 to 15.7). The pubertal status at the time of the first transplantation was stage 1 in twelve patients and stage 2 or more in eight patients, according to Tanner and Whitehouse.[8] Eleven patients started puberty later than the 90 % confidence interval for Dutch children and nine patients started puberty within normal age limits.[9]

The number of transplantations varied from one to four. Eleven patients received more than one renal transplantation. The time since the first transplantation varied from 9.1 to 17.8 years (mean 14.6 years). Three patients did not have a functioning renal transplant at the time of the BMD measurement; two were treated with haemodialysis (patients 14 and 20 in Table 1) and one with peritoneal dialysis (patient 11).

The underlying renal disease was chronic glomerulonephritis in 10 patients, congenital renal hypoplasia in four, chronic pyelonephritis in two, medullary cystic disease in two (brother and sister), nephronophtisis in one and an unclassified granulomatous nephritis in one patient. Seven patients had a congenital renal disease.

*Medication*

The standard immunosuppressive drug regimen after renal transplantation included corticosteroids in all patients. Acute rejection episodes were generally treated with three to five alternate day infusions of methylprednisone (20 mg/kg). At the time of the study eighteen patients were on prednisone treatment, four on alternate days and 14 daily, with doses ranging from 2 mg on alternate days to 10 mg daily.

The individual cumulative dose of prednisone/kg bodyweight was calculated from the time of the first renal transplantation until the BMD measurement. The total duration of prednisone therapy from the first renal transplantation until the BMD measurement, the percentage of time on alternate day prednisone therapy, the number of acute rejection episodes and the total duration of dialysis before and between renal transplantations were determined.

*Measurements*

The height was measured by a fixed wall stadiometer and expressed as standard deviation score (SDS) compared to Dutch sex and age matched reference values.[9] The

body mass index was calculated as weight divided by square height ($kg/m^2$) and was also expressed as SDS.[10]

The BMD of the lumbar vertebrae L2-L4 and of the total body was measured by Dual Energy X-ray Absorptiometry (DXA, model DPX-L, Lunar Corporation).[11] BMD is expressed as g hydroxyapatite/$cm^2$. The coefficient of variation (SD) was 1.1 (0.2)%. The BMD results of the lumbar spine and total body were converted to a SDS by comparing the results to a British reference population of sex and age matched young adults, provided by Lunar Corporation. This reference group was used in the statistical analysis. The British lumbar spine reference data did not differ from lumbar spine BMD measurements of a smaller Dutch reference sample.[12]

Approval of the ethics Committee of the University Hospital Rotterdam and informed consent of the participants of the study were obtained.

*Statistical analyses*

Student's t-tests were used to compare intergroup differences. We used simple linear regression analysis to assess a correlation between a continuous factor and BMD SDS. Spearman's rank correlation coefficient was used for covariables that were not normally distributed. Multiple regression analysis (stepwise forward selection) was performed to determine the effect of the factors which had a significant effect in univariate analysis on BMD SDS simultaneously. All tests were performed for both lumbar spine and total body BMD SDS.

## Results

Fourteen patients (70%) had a SDS of the BMD of the lumbar spine below -1, of whom six were below -2. The mean SDS of the BMD of the lumbar spine of the patients was -1.7 (range -4.1 to 0.2), which is significant below zero ($p<0.001$). Fifteen patients (75%) had a SDS of the BMD of the total body below -1, of whom seven patients were below -2. The mean SDS of the BMD of the total body was -1.6 (range -3.9 to 0.8), significant below zero ($p<0.001$).

The individual results of the 20 patients are summarized in Table 1. The correlation coefficient between BMD SDS of lumbar spine and total body was 0.80 ($p<0.001$). Twelve patients had a BMD SDS below -1 of lumbar spine as well as total body, five patients had a BMD SDS below -2 of both measurements.

The results of simple linear regression analyses are summarized in Table 2. The relation between cumulative dose of prednisone and BMD SDS is illustrated in the Figure.

**Table 1**

*Clinical data of 20 adult patients after renal transplantation in childhood and results of bone mineral density (BMD) measurements.*

| Patient number | Sex | Age (yrs) | Primary disease * | Age at first renal transplant | No. of transpl. | Final height SDS | Body mass index SDS | Cum. dose of predn. | BMD spine SDS | BMD total body SDS |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | F | 28 | CGN | 11.5 | 1 | -0.5 | -0.1 | 1079 | -1.9 | -0.7 |
| 2. | M | 26 | CPN | 9.7 | 1 | -4.5 | -1.1 | 1558 | -1.8 | -2.4 |
| 3. | M | 24 | CHP | 9.2 | 1 | -2.4 | 2.2 | 818 | -0.4 | 0.0 |
| 4. | M | 23 | CGN | 7.1 | 1 | -0.9 | -0.8 | 1059 | -1.9 | -2.0 |
| 5. | M | 24 | CPN | 7.7 | 1 | -0.9 | 0.0 | 1182 | -1.5 | -1.7 |
| 6. | M | 28 | CGN | 12.1 | 1 | -1.6 | -0.4 | 1795 | -3.7 | -3.1 |
| 7. | F | 26 | CHP | 15.0 | 1 | -1.5 | 0.5 | 610 | 0.2 | -1.1 |
| 8. | M | 24 | NN | 14.5 | 1 | -2.5 | 1.6 | 553 | -2.6 | -1.6 |
| 9. | M | 23 | CGN | 13.7 | 1 | 0.8 | 0.9 | 483 | -0.7 | -0.3 |
| 10. | M | 28 | CGN | 10.7 | 2 | -5.1 | -0.6 | 2166 | -4.1 | -3.9 |
| 11. | M | 26 | CHP | 11.6 | 2 | -3.1 | -0.8 | 1081 | -1.6 | -1.8 |
| 12. | F | 24 | CGN | 10.5 | 2 | -3.6 | -1.3 | 1364 | -2.9 | -2.5 |
| 13. | M | 26 | CHP | 15.7 | 2 | -4.6 | 1.6 | 591 | -1.8 | -1.0 |
| 14. | F | 28 | MCD | 11.3 | 3 | -4.7 | -0.2 | 1847 | -2.1 | -3.3 |
| 15. | F | 29 | CGN | 14.5 | 3 | -2.1 | 1.2 | 859 | -0.2 | 0.8 |
| 16. | M | 23 | GR | 12.6 | 3 | -4.2 | 0.5 | 1186 | -1.7 | -1.3 |
| 17. | M | 27 | CGN | 15.1 | 3 | -3.7 | -0.3 | 1191 | -2.8 | -3.5 |
| 18. | F | 21 | CGN | 11.9 | 3 | -2.8 | 0.1 | 656 | -1.6 | -1.9 |
| 19. | F | 25 | CGN | 14.1 | 3 | 0.8 | -0.3 | 706 | -0.2 | -0.1 |
| 20. | M | 26 | MCD | 12.9 | 4 | -2.5 | -1.1 | 1952 | -0.4 | -1.7 |

Cum.dose of predn. = cumulative dose of prednisone (mg/kg body weight).

* CGN= chronic glomerulonephritis, CPN= chronic pyelonephritis, CHP= congenital renal hypoplasia, NN= nephronophtisis, MCD= medullary cystic disease, GR= granulomatous nephritis

SDS = standard deviation score

**Table 2**

*Correlation between cumulative dose of prednisone, final height, body mass index and bone mineral density (BMD) standard deviation score (SDS).*

| | Lumbar spine BMD SDS | Total body BMD SDS |
|---|---|---|
| cumulative dose of prednisone | -0.53** | -0.70*** |
| final height SDS | 0.47* | 0.56** |
| body mass index SDS | 0.34 | 0.59** |

* $p<0.05$ ; ** $p<0.01$; *** $p<0.001$.

The equation for the BMD SDS of the total body was as follows: BMD SDS =0.337 - 1.74 (SE 0.41) x cumulative dose of prednisone (g/kg), which means that one g prednisone/kg bodyweight reduced the SDS of the total body BMD with 1.74. For the lumbar spine BMD SDS this reduction was 1.23 (SE 0.47). There was no correlation between the BMD SDS and the percentage of time on alternate day prednisone treatment. Although the mean final height SDS was markedly reduced (mean -2.5), the mean body mass index SDS did not differ from average (mean 0.1). Thirteen patients (65%) had a height SDS below -2. There was a significant positive correlation between height SDS and BMD SDS of both measurements and between body mass index SDS and total body BMD SDS.

The patients who received the first renal transplantation at Tanner stage 1 (n=12) had a lower mean (SE) BMD SDS than the patients who had started puberty at the time of the first renal transplantation: -2.12 (0.30) *v* -0.93 (0.45), p = 0.032 for total body BMD SDS and -1.97 (0.33) versus -1.25 (0.41) for lumbar spine BMD SDS. There is, however, a significant difference in cumulative dose of prednisone between the two groups.

The 11 patients with delayed puberty had a lower mean (SE) BMD SDS than the nine patients who had started puberty at normal time, -2.03 (0.38) and -1.16 (0.38) respectively for the total body, -2.01 (0.39) and -1.27 (0.32) respectively for the lumbar spine, although this difference was not statistically significant.

Factors without significant effect on BMD SDS in a simple linear regression model were: gender, number of transplantations, dialysis at the time of the measurement, age at first renal transplantation, years since the transplantation, duration of dialysis, current

prednisone dose, total duration of prednisone treatment, total number of acute rejection episodes, and either acquired or congenital renal disease.

Stepwise forward selection with BMD SDS of the total body or lumbar spine as dependent variable resulted in a model only containing cumulative dose of prednisone. The pubertal stage at the time of the transplantation, delayed puberty, body mass index SDS and height SDS had no additional significant effect on BMD SDS.

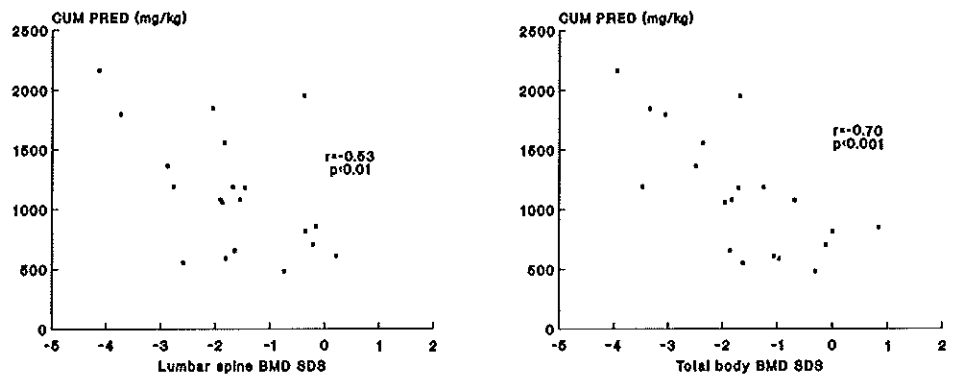


**Figure 1**

*Relation between cumulative dose of prednisone (CUM PRED) and bone mineral density (BMD SDS). r= correlation coefficient.*

## Discussion

Our study indicates that most young adult patients who had received a renal transplantation in childhood had moderately to severely decreased BMD. This is the first study with such a long follow-up.

These patients were aged between 20 and 30 years at the time of the study. Healthy individuals have their maximal BMD at this age.[6] It has been reported that every 1 standard deviation (SD) reduction in bone mass gives an approximate doubling of fracture risk in post-menopausal women.[13] Differences of 2 SD in bone mass of the lumbar spine, radius or calcaneus were associated with fourfold to sixfold increases in the risk for vertebral fractures in post-menopausal women.[14]

Thirty percent of our patients had significant osteopenia of the lumbar spine, BMD more than 2 SD below the mean, and 35% of the patients had osteopenia of the total body. The lumbar spine is mainly composed of trabecular bone and the bone of the total body is mainly composed of cortical bone (80% cortical bone versus 20%

trabecular bone).[15] As corticosteroid-induced osteoporosis mainly affects trabecular bone[16-19] and hyperparathyroidism mainly affects cortical bone[20] both these factors appear to be involved in the osteopenic process.

Other studies reported significant bone loss directly after renal transplantation in adult patients.[2,3] Progressive loss of BMD of the lumbar spine and femoral neck, measured by DXA, was found in 26 adult patients during the first six months after renal transplantation.[2] Likewise Julian *et al.* found that the mean BMD of the vertebrae, measured by dual-photon absorptiometry, of twenty adult patients had decreased 8.8 percent eighteen months after transplantation.[3] The mean spinal BMD was 1.17 SD below normal at the time of transplantation. In this study the BMD of the radial shaft, mostly cortical bone, had increased significantly six months after transplantation from -0.7 to -0.3 SDS. This may reflect a resolution of secondary hyperparathyroidism. This increase is in contrast to what was found by Kwan *et al.*[2] We measured bone mass nine to 17 years after renal transplantation and found that the mean SDS for cortical BMD was -1.6, significantly below controls.

Significant osteopenia of the radius, ulna, and humerus was measured in 11 of 18 children, aged 4 till 18 years, who had received a renal transplant six months to eight years before, using single photon absorptiometry.[4]

Glucocorticoid induced osteopenia is the result of a number of factors that adversely affect calcium homeostasis. Glucocorticoids decrease bone formation and increase bone resorption[16,17], mainly of trabecular bone. Intestinal calcium absorption is reduced and renal calcium excretion is increased. We found a significantly negative correlation between the cumulative dose of prednisone and both lumbar and total body BMD. Also Dykman *et al.* found that the effect of long-term glucocorticoid therapy in various regimens may result in glucocorticoid-induced osteopenia and this effect was cumulative.[18] Both daily[16,19] and alternate day[21] corticosteroid therapy cause bone loss. However, Chesney et al found that the children receiving daily prednisone treatment had a significantly lower bone mineral content than those receiving alternate day treatment.[4]

In addition to renal osteodystrophy and corticosteroids a delay of puberty may contribute to the development of post-renal transplantation osteopenia. Late puberty is common in children after renal transplantation.[22] In our study 11 of the 20 patients (55%) had a late start of puberty and their BMD SDS tended to be lower than the patients who started puberty at a normal age. Bone mass increases with age throughout childhood reaching the peak bone mass by late adolescence or early adulthood.[23-26] Bone mass accretion during puberty appears to be critical in the development of peak bone mass.[27] Peak bone mass is regarded as a major determinant of osteoporosis in later life.[7] Healthy men who had late puberty (the onset of puberty after 15 years of

age) had a significant lower BMD of the radius and lumbar spine than men who had their puberty at a normal age.[28]

In 65% of the patients of this study the final height was more than 2 SD below the mean. This is in accordance with the study of Hokken-Koelega et al showing that in 77% of the males and 71% of the females the final height remained below the third percentile (< -1.88 SD).[29] These patients received their first renal transplant before the age of 15.

We found a positive correlation between the height SDS and BMD SDS. Growth may be influenced by the same factors as the bone mass. Some authors described that the method of DXA to measure BMD was influenced by bone size and by calculating the volumetric density, using hypothetical models of the vertebral column, the confounding effect of bone size could be reduced.[30,31] However, Mazess et al demonstrated that the BMD measured by dual photon absorptiometry, which differed from DXA only in the radiation source, correlated well with the quantitative computer scan density of both the total vertebrae (r=0.89) and that of the entire body (r=0.86).[32] The quantitative computer scan measures the volumetric density. In a study of total body BMD in normal women multiple linear regression revealed that height did not have a statistically significant influence on the BMD.[33] We propose that the correlation between height and BMD cannot be explained by the technique used but that both BMD and height were negatively influenced. Multiple regression analysis revealed that the cumulative dose of prednisone had the most important negative influence on BMD. This may have influenced the final height as well.

We found a significant positive correlation between the body mass index SDS and total body BMD SDS. In healthy adults weight and body mass index had a positive effect on femur and spine BMD[34], probably due to load on weight-bearing bones.

Our results showed that patients who had received a renal transplantation in childhood had a significantly reduced BMD. In multiple regression analysis only the cumulative dose of prednisone appeared to have a significant effect on BMD. Children who receive a renal transplantation have a highly increased risk of developing osteoporosis. Glucocorticoid sparing immunosuppressive regimens might reduce osteopenia after transplantation.

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# Chapter 7.2

# Bone mineral density, bone metabolism and body composition of children with chronic renal failure, with and without growth hormone treatment

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## Abstract

Osteopenia has been reported in adult patients with chronic renal failure (CRF). Only a few studies have been performed in children. Children with CRF and growth retardation can be treated with growth hormone (GH). GH affects both growth and bone metabolism. The objective of this study was to evaluate bone mineral density (BMD), bone turnover, body composition in paediatric patients with CRF and to study the effect of GH on these parameters.

Thirty-six prepubertal patients (27 boys and 9 girls), mean age 7.9 years, with CRF participated in the study. Two groups were identified : patients with growth retardation who received GH (GH-group) and patients who did not receive GH and of whom most of them were not growth retarded (no-GH-group). After an observation period of 6 months, the seventeen patients of the GH-group started GH treatment. Fourteen completed one year treatment. The no-GH-group consisted of nineteen patients, of whom 16 were followed for 6 months, 14 for 12 months and 13 of them for 18 months. Lumbar spine BMD, total body BMD and body composition were assessed by dual energy x-ray absorptiometry every 6 months, compared to age- and sex-matched reference values of the same population and expressed as standard deviation scores (SDS). BMD of appendicular bone was measured by quantitative microdensitometry (QMD). Blood samples were obtained to assess bone metabolism.

At baseline mean lumbar spine and total body BMD SDS of all patients were not significantly different from zero. Mean lumbar spine and total body BMD SDS did not change significantly in the GH-group during GH treatment. The increase of QMD rho50 during the first 6 months of GH treatment was significantly less than during the observation period ($p<0.01$). Height SDS and biochemical markers of both bone formation and bone resorption increased significantly during GH treatment; 1,25-dihydroxyvitamin D remained stable. Lean tissue mass increased ($p<0.001$) and percentage body fat decreased ($p<0.01$) during GH treatment. BMD, the biochemical markers of bone turnover which are independent of renal fuction, and body composition remained stable in the no-GH-group.

In conclusion, mean lumbar spine and total body BMD of children with CRF did not differ from healthy controls. Growth and bone turnover increased and body composition changed during GH treatment. BMD remained stable, in the GH-group as well as in the no-GH-group. Adequate treatment with alpha-calcidol might have prevented osteopenia in these patients.

## Introduction

Metabolic bone disease is frequently observed in chronic renal failure (CRF). Different histological lesions of renal osteodystrophy have been reported in paediatric patients with CRF.[1] The long-term consequence of the bone disease is progressive osteopenia in most cases. Children with osteopenia have a higher risk of fractures in childhood and may have a higher risk to develop osteoporosis later in life because of not reaching their optimal peak bone mass. Peak bone mass and subsequent bone loss are the major determinants of osteoporosis at older age. A high peak bone mass provides a larger reserve later in life.[2]

In adults, decreased bone mineral density (BMD) was observed in patients with CRF.[3-5] Both appendicular (predominantly cortical bone) and axial (predominantly trabecular bone) BMD related to the degree of renal failure.[3] Parathyroid hormone (PTH) levels were inversely correlated with appendicular BMD.[3] A significant decline of BMD of the forearm was observed in 30 of 74 children with renal diseases.[6] Another study reported no decrease in spinal BMD in a small group of children with CRF.[7] After renal transplantation in childhood, most young adult patients had moderate to severe osteopenia.[8]

Treatment with recombinant human growth hormone (GH) has been shown to enhance growth rate in growth-retarded children with CRF.[9,10] In addition to its effects on linear growth, GH influences bone and mineral metabolism.[11-13] Bone turnover and BMD increased during GH therapy in children as well as in adults with GH deficiency.[14-18]

The objective of the present study was to evaluate BMD, bone turnover, body composition, growth and growth factors in paediatric patients with CRF during GH treatment and in children with CRF without GH treatment.

## Patients and methods

Thirty-six prepubertal patients, 27 boys and 9 girls, entered the study. The mean age was 7.9 years (range 3 to 12 years). Written informed consent was obtained from the parents. The study was approved by the ethics committee of each participating centre.

Inclusion criteria were : (1) calculated creatinine clearance below 60 ml/min per 1.73 $m^2$; (2) no clinical evidence of puberty (Tanner stage I)[19]; (3) normal thyroid function; (3) no endocrine or metabolic disease (except renal); (4) no treatment with corticosteroids within the last 6 months; (5) no oxalosis or cystinosis.

Two groups were identified : children with CRF and growth retardation who got treatment with GH (GH-group) and children with CRF who did not receive GH

treatment, most of them did not have growth retardation (no-GH-group). Growth retardation was defined as height standard deviation score (SDS) for chronological age less than -1.88[20] and growth velocity below the 50th percentile[19] or height SDS below zero and growth velocity below the 25th percentile.

The underlying renal disease was congenital renal dysplasia in 9 patients (5 of GH-group), obstructive nephropathy in 7 patients (2 of GH-group), reflux nephropathy in 7 patients (3 of GH-group), prune belly syndrome in 2 patients (1 of GH-group), neurogenic bladder (without other neurological symptoms) in 2 patients (1 of GH-group), polycystic kidney disease in 2 patients, chronic glomerulonephritis in 3 patients (2 of GH-group), interstitial nephritis in 1 patient (of GH-group), glomerulosclerosis in 1 patient (of GH-group), haemolytic uraemic syndrome in 1 (of GH-group), and kidney damage after shock in 1 patient.

All but one patient received alpha-calcidol (1α hydroxyvitamin D), which is metabolised in the liver to 1,25-dihydroxyvitamin D. The median dose in the GH-group was 0.40 μg/day and in the no-GH-group 0.25 μg/day at baseline. The dose was adjusted according to renal function, levels of PTH and total serum calcium. In the GH-group, the dose increased in 4 patients, decreased in 1 patient and both increased and decreased in 3 patients during GH treatment. In the no-GH-group the dose increased in 3 patients, decreased in 2 patients and consequently increased and decreased in 3 patients. Eight patients had dialysis (7 of the GH-group).

The GH-group (n=17) had an observation period of 6 months. Thereafter, they received one subcutaneous injection per day of biosynthetic human GH (Norditropin, Novo Nordisk A/S) 4 IU/m$^2$ body surface during 1 year. All 17 completed 6 months treatment, 14 completed 12 months treatment. Three dropped out of the study because of renal transplantation.

The no-GH-group consisted of 19 patients, of whom 16 were followed for 6 months, 14 for 12 months and 13 for 18 months.

Patients were examined at baseline and every three months. Data for the study were obtained every six months. Now we report the follow-up till 18 months study. Height was measured with a Harpenden stadiometer and expressed as SDS.[20]

Lumbar spine and total body bone mineral density was assessed by dual energy-x-ray absorptiometry (DXA, Lunar, DPXL/PED, Wisconsin, USA). All children were measured on the same apparatus. The results were compared to our age- and sex-matched Dutch reference values (n=500, 4 to 20 years of age)[21] and expressed as SDS. The coefficient of variation of spinal BMD measurement was 1.1 (0.2) % in our setting. With the total body measurement by DXA the body composition was measured as lean tissue mass, fat mass and bone mineral content. The coefficients of variation have been reported as 2.2 % for fat mass, 1.1 % for lean tissue mass and 0.6 % for

bone mineral content.[22] Bone mineral content, lean tissue mass, fat mass and percentage body fat were compared to our age- and sex-matched Dutch reference values (4 to 20 years old) and expressed as SDS.[23]

BMD of appendicular bone was assessed by quantitative microdensitometry (QMD). This technique has been described before.[24] It uses an X-ray of the left hand. QMD measurements were performed on the middle phalanx of the index finger. The BMD is expressed as rho. At a distance of 25 % from the proximal metaphyseal end (rho25) around 60 % of the bone is trabecular, while at a distance of 50 % from the proximal metaphyseal end (rho50) around 80 % is cortical bone.[24] Reference data of healthy children were used.[24]

Blood samples for the study were obtained in the morning every 6 months and once extra after 3 months GH treatment in the GH-group for the assessment of urea, creatinine, calcium, magnesium, phosphate, alkaline phosphatase, 1,25-dihydroxyvitamin D, PTH, osteocalcin and procollagen type I C-terminal propeptide (PICP), carboxy-terminal telopeptide of type I collagen (ICTP), insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein 3 (IGFBP-3). Osteocalcin was measured by radioimmunoassay (Incstar Corporation, Stillwater, USA), immunoreactive PTH by radioimmunoassay (Nichols Institute, San Juan Capistrano, CA, USA) and 1,25-dihydroxyvitamin D by radioimmunoassay of Immuno Diagnostic System (Boldon, United Kingdom). For measurement of PICP and ICTP radioimmunoassay kits of Orion Diagnostica, Espoo, Finland were used. Our own reference values of prepubertal healthy children were used for osteocalcin, PICP and ICTP.[25] Assessment of IGF-I and IGFBP-3 was performed with radioimmunoassay kits of Med-Genix Diagnostics, Fleurus, Belgium and Diagnostic System Laboratories, Webster, Texas. IGF-I sex- and age-matched reference values were based on 600 samples of a healthy Dutch population.[26]

Acid-base balance was checked every three months. None of the patients had chronic acidosis, defined as capillary bicarbonate below 20 mmol/l in more than one measurement.

*Statistics*

One sample t-tests were used to compare the mean SDS of the patients with the expected zero. The within patient change was tested with an one sample t-test to compare with zero. The means of two groups of variables with a normal distribution were compared with two sample t-tests. This test was also used to compare the change in QMD during GH with the change during the observation period. Pearson's correlation coefficient was calculated to test an association between variables with a normal distribution and Spearman's rank correlation coefficient in case of a non-normal

distribution.

## Results

*Bone mass*

At baseline, the means of lumbar spine BMD SDS and of total body BMD SDS of all patients were not significantly different from zero. Mean lumbar spine BMD SDS was 0.35 (sd 1.41) and mean total body BMD SDS was -0.07 (1.68). None of the patients had lumbar spine BMD SDS below -2, and 4 patients (13%) had total body BMD SDS below -2, all of the no-GH-group. There was no significant difference in lumbar spine or BMD SDS between the GH-group and no-GH-group. No BMD SDS could be calculated of five patients who were younger than 4 years of age because our reference data start from the age of 4 years. During the 6 months observation period lumbar spine and total body BMD SDS remained stable (Table 1).

In the GH-group, lumbar spine and total body BMD SDS had not changed significantly after 6 and 12 months of GH therapy (Table 1). After 6 months, mean change of ($\Delta$) lumbar spine BMD SDS was -0.16 (sd 0.91) and of total body BMD SDS -0.19 (sd 0.58). During 12 months of GH treatment lumbar spine BMD SDS increased on average 0.48 (sd 1.18) and total body BMD SDS decreased on average -0.32 (sd 0.59). After 1 year of therapy none of the GH-group children had lumbar spine or total body BMD SDS below -2. The changes in lumbar spine or total body BMD SDS were not significantly different between the children on dialysis and the ones without dialysis.

In the no-GH-group, lumbar spine BMD SDS and total body BMD SDS remained stable (Table 1). There was no significant difference in the change of lumbar spine or total body BMD SDS between the GH-group and no-GH-group.

Appendicular bone was decreased at baseline in 2 patients (1 GH group) when measured at rho25 and in 2 other patients (GH-group) when measured at rho50. The change in rho50 during the first 6 months of GH therapy was significantly smaller than the change in rho50 during the 6 months observation period ($p<0.01$). It was also smaller than the change in rho50 of the no-GH-group over the same period ($p=0.01$). The change in rho25 during the first 6 months of GH therapy was similar as during the observation period. The change in rho50 or rho25 between 6 and 12 months GH treatment was not significantly different from the change in the observation period.

*Height*

Mean height SDS was significantly lower than zero in the GH-group ($p<0.001$) as well as in the no-GH-group ($p<0.001$) at the first measurement. Height SDS was

**Table 1**

*Mean (sd) of standard deviation score (SDS) of bone mineral density (BMD, g/cm²), bone mineral content (BMC, g), body composition, height, and Quantitative Microdensitometry (QMD) of patients with chronic renal failure treated with growth hormone (GH-group) and not treated with GH (no-GH-group). The numbers of patients of whom a DXA measurement was performed are given. The within patient change was tested compared to t=0.*

| | GH-GROUP | | | | NO-GH-GROUP | | | |
|---|---|---|---|---|---|---|---|---|
| | t=-6 months | t=0 start GH | t=6 months GH | t=12 months GH | t=-6 months | t=0 | t=6 months | t=12 months |
| | n=16 | n=16 | n=16 | n=13 | n=15 | n=14 | n=14 | n=13 |
| lumbar spine BMD SDS | -0.04 (1.38) | 0.31 (1.42) | 0.15 (1.22) | 0.93 (1.42) | 0.77 (1.37) | 0.32 (1.30) | 0.37 (1.23) | 0.19 (1.21) |
| total body BMD SDS | -0.17 (1.40) | 0.15 (1.02) | -0.04 (1.03) | -0.18 (1.26) | 0.03 (1.99) | -0.22 (1.74) | -0.24 (1.58) | -0.24 (1.41) |
| total body BMC SDS | -1.58 (0.88) | -1.47 (0.85) | -1.36 (0.83) | -1.09 (0.88) | -0.85 (1.22) | -1.03 (1.11) | -1.03 (1.04) | -1.07 (0.98) |
| lean tissue mass SDS | -2.03 (0.79) | -1.98 (0.88) | -1.58 (1.03)[3] | -1.09 (1.08)[3] | -1.12 (0.75) | -1.19 (0.63) | -1.29 (0.71) | -1.27 (0.62) |
| fat mass SDS | -0.29 (0.48) | -0.28 (0.51) | -0.53 (0.41)[1] | -1.08 (0.75)[3] | -0.06 (0.65) | -0.15 (0.71) | -0.20 (0.78) | -0.75 (1.04) |
| % body fat SDS | -0.06 (1.08) | -0.05 (1.08) | -0.86 (0.96)[2] | -0.95 (0.94)[3] | 0.11 (1.00) | -0.09 (1.15) | -0.24 (1.26) | -0.54 (1.17) |
| height SDS | -2.29 (0.49) | -2.31 (0.53) | -1.92 (0.58)[3] | -1.46 (0.63)[3] | -1.28 (1.04) | -1.19 (0.95) | -1.12 (0.93) | -1.14 (1.02) |
| QMD rho50 (g/cm³) | 0.94 (0.09) | 0.99 (0.09) | 0.96 (0.08)* | 0.98 (0.09) | 0.96 (0.11) | 0.93 (0.10) | 0.97 (0.11) | 1.00 (0.10) |
| QMD rho25 (g/cm³) | 0.69 (0.06) | 0.75 (0.09) | 0.78 (0.10) | 0.79 (0.10) | 0.68 (0.08) | 0.68 (0.07) | 0.70 (0.10) | 0.72 (0.06) |

[1] $p<0.05$, [2] $p<0.01$, [3] $p<0.001$ significant change compared to t=0.

* the mean within patient change in rho50 between 0 and 6 months was significantly smaller than the change between -6 and 0 months, $p<0.01$.

significantly lower in the GH-group than in the no-GH-group (p=0.001). Height SDS did not change significantly during the observation period. Height SDS increased significantly during GH treatment in the GH-group (Table 1). Height SDS remained stable in the no-GH-group.

*Body composition*

Mean lean tissue mass SDS and bone mineral content SDS of all patients was significantly lower than zero at baseline (mean -1.59 (sd 1.03), respectively -1.22 (1.10), both p<0.001). Mean percentage body fat and fat mass SDS were not significantly different from zero. Only lean tissue mass SDS was significantly lower in the GH-group than in the no-GH-group (p<0.01). During GH treatment, lean tissue mass SDS increased, whereas percentage fat and fat mass SDS decreased significantly (Table 1). Bone mineral content SDS did not change significantly. Lean tissue mass, bone mineral content, percentage body fat, and fat mass SDS did not change significantly in the no-GH-group during the study period (Table 1).

*Biochemical bone parameters and growth factors*

The results of biochemical bone parameters and growth factors are shown in Table 2. Mean osteocalcin, ICTP and PICP were significantly higher than those of healthy prepubertal children (p<0.001 for osteocalcin and ICTP and p<0.02 for PICP). Mean IGF-I SDS was significantly lower than normal in the GH-group (p<0.05) and did not differ from normal in the no-GH-group. Two children had high IGFBP-3 (1 in GH-group). During the observation period all biochemical parameters remained stable (Table 2). After 3 months GH treatment osteocalcin, PICP, ICTP, alkaline phosphatase, IGF-I, IGF-I SDS, and IGFBP-3 had increased significantly (p<0.05 for osteocalcin and alkaline phosphatase, p<0.02 for PICP and p<0.001 for ICTP, IGF-I, IGF-I SDS, and IGFBP-3) (data not shown). After 6 and 12 months of GH therapy, osteocalcin, PICP, ICTP, alkaline phosphatase, IGF-I, IGF-I SDS, and IGFBP-3 had increased significantly when compared to baseline (Table 2). One boy entered puberty during the study period. The results did not change if this child was excluded.

In the no-GH-group osteocalcin, IGF-I, IGF-I SDS, and IGFBP-3 increased (Table 2). Three children of the no-GH-group entered puberty during the study period. IGF-I SDS remained stable if these children were excluded. The increase of the other variables was still significant. The increase of osteocalcin and IGFBP-3 was significantly smaller in the no-GH-group than in the GH-group (respectively p=0.02 and p=0.01). Creatinine clearance decreased slightly in the no-GH-group.

Calcium, phosphate, urea, magnesium, PTH and 1,25-dihydroxyvitamin D did not change significantly during the study period. The change in 1,25-dihydroxyvitamin D

**Table 2**

*Biochemical markers of bone metabolism and growth factors, mean (sd), in children with chronic renal failure. GH-group represents the children treated with growth hormone started at t=0. No-GH-group represents the children not treated with growth hormone. The median is given of PTH. alk. phosph.= alkaline phosphatase, PTH= parathyroid hormone, 1,25 vit.D= 1,25-dihydroxyvitamin D, PICP= procollagen type I C-terminal propeptide; ICTP = carboxy-terminal telopeptide of type I collagen, IGF-I = insulin-like growth factor-I, SDS = standard deviation score, IGFBP-3= insulin-like growth factor binding protein 3. The calculated creatinine clearance is presented of the patients without dialysis. The within patient change was tested compared to t=0.*

| | | GH-GROUP | | | | NO-GH-GROUP | | | |
|---|---|---|---|---|---|---|---|---|---|
| | refe-rence values | t=-6 months n=17 | t=0 start GH n=17 | t=6 months GH n=17 | t=12 months GH n=14 | t=-6 months n=19 | t=0 n=16 | t=6 months n=14 | t=12 months n=13 |
| osteocalcin (µg/l) | 4-20 | 34 (17) | 29 (15) | 41 (15)[2] | 49 (25)[3] | 32 (10) | 29 (10) | 30 (16) | 33 (10)[4] |
| PICP (µg/l) | 77-626 | 443 (142) | 362 (131) | 498 (166)[1] | 467 (183)[2] | 413 (190) | 409 (173) | 341 (146) | 437 (145) |
| ICTP (µg/l) | 6-19 | 72 (71) | 59 (44) | 106 (66)[4] | 110 (83)[3] | 52 (35) | 52 (51) | 53 (55) | 50 (38) |
| alk. phos. (U/L) | 80-225 | 143 (57) | 141 (88) | 322 (208)[3] | 308 (236)[1] | 180 (69) | 160 (92) | 165 (81) | 192 (112) |
| PTH (ng/l) | 10-55 | 46 | 45 | 55 | 89 | 45 | 47 | 54 | 70 |
| 1,25-OHD (pmol/l) | 39-102 | 78 (57) | 80 (49) | 77 (61) | 105 (60) | 89 (54) | 101 (46) | 91 (54) | 93 (56) |
| IGF-I (nmol/l) | | 19 (15) | 21 (16) | 48 (20)[4] | 54 (31)[4] | 23 (15) | 22 (14) | 35 (26)[1] | 36 (27)[2] |
| IGF-I SDS | | -1.16 (1.89) | -1.15 (1.95) | 1.59 (1.89)[4] | 1.61 (1.70)[4] | -0.53 (1.32) | -0.76 (1.64) | 0.02 (1.07)[1] | 0.08 (1.13)[1] |
| IGFBP-3 (mg/l) | | 3.6 (1.8) | 3.1 (2.0) | 5.3 (1.5)[4] | 5.3 (1.5)[4] | 3.7 (1.40 | 3.4 (1.6) | 4.3 (1.6)[4] | 4.1 (1.2)[4] |
| creatinine clearance (ml/min per 1.73 m$^2$) | | 25 (18) | 26 (14) | 28 (23) | 33 (25) | 35 (15) | 37 (18) | 35 (19)[1] | 34 (20) |

[1] $p<0.05$, [2] $p<0.02$, [3] $p<0.01$, [4] $p<0.001$ significant increase compared to t=0.

did not differ between the patients on dialysis and the ones not on dialysis.

*Correlations*

At the first measurement, height SDS correlated positively with lumbar spine BMD SDS ($r=0.44$, $p<0.02$) and had no significant correlation with total body BMD SDS. Creatinine clearance correlated negatively with osteocalcin and ICTP ($r=-0.67$ and $r=-0.59$, respectively, both $p<0.001$). PTH correlated with osteocalcin ($r=0.58$, $p<0.001$).
In the GH-group, the Δ of height SDS had no significant correlation with the Δ of lumbar spine or total body BMD SDS, or of the biochemical markers of bone turnover or of the growth factors.

## Discussion

Mean lumbar spine and total body BMD of the 36 paediatric patients with CRF did not differ from healthy controls. During GH treatment, BMD did not change significantly in the growth-retarded patients, while height SDS, growth factors and bone turnover increased. During the first 6 months of GH treatment a small decrease was seen in the QMD rho50. Lean tissue mass increased and fat mass decreased during GH treatment. BMD, body composition, height, and the markers of bone turnover which are independent of renal function remained stable in the patients with CRF not treated with GH.
In adults BMD has shown to be negatively related to the duration of CRF.[4] In the present study no decrease in BMD was found. This may be related to the shorter duration of CRF or to adequate treatment with vitamin D which may have prevented renal osteodystrophy and osteopenia.
GH increased growth velocity but seemed not to affect BMD of the growth-retarded patients with CRF. Children with GH deficiency have growth retardation and decreased BMD and both height and BMD improve during GH therapy in these children.[15-17] Several factors may have contributed to this discrepancy. Mean BMD of the growth retarded patients with CRF at baseline was not decreased, in contrast to BMD of children with GH deficiency. Secondly, serum levels of GH are not decreased in children with CRF but various measures of the tissue response to GH are diminished in patients with CRF.[27] Thirdly, in the present study levels of 1,25-dihydroxyvitamin D remained stable during GH treatment. It is known that 1,25-dihydroxyvitamin D stimulates intestinal calcium transport and promotes the mineralization of osteoid laid down by osteoblasts by maintaining the calcium and phosphorus concentrations within the normal range.[28] Furthermore, 1,25-dihydroxyvitamin D enhances the synthesis of

osteocalcin by osteoblasts indicating direct stimulatory effects on these cells.[29,30] 1,25-dihydroxyvitamin D is primarily synthesized in the kidney. GH increases its production by stimulating renal 1α hydroxylase activity through IGF-I.[11,31] In contrast to observations in GH-deficient subjects, our patients with CRF did not show an increase in serum 1,25-dihydroxyvitamin D levels during GH treatment.[15,16,18] Based on these findings we hypothesize that the lack of a GH-induced increase in 1,25-dihydroxyvitamin D levels might be linked to the absence of a response in BMD. This hypothesis is supported by the reported observation of a positive correlation between the increase in BMD and 1,25-dihydroxyvitamin D and between the increase of IGF-I and 1,25-dihydroxyvitamin D in GH-deficient children during GH treatment.[16] The increase of 1,25-dihydroxyvitamin D may be important for the stimulatory action on osteoblastic activity of GH. The absence of an increase of 1,25-dihydroxyvitamin D in our patients with CRF during GH treatment may be due to the renal failure, the high bone turnover but above all the treatment with alpha-calcidol of these patients.

Our results are in agreement with findings of Zadik *et al.*[7] who reported no significant increase in spinal BMD after one year of GH treatment in children with CRF.

Bone turnover increased during GH treatment as is also reported in children and adults with growth hormone deficiency.[14-16,18] In growing children modeling of new bone as well as remodeling of existing mineralized tissue are ongoing processes. It is likely that these two processes are controlled by different sets of hormonal mediators and /or that the actions of hormones differ in chondrocytes and osteoblasts.[27] The biochemical markers are not specific for either bone modeling or skeletal remodeling.[27] In healthy children biochemical bone markers increase in periods of increased height velocity.[32] The increase in bone turnover during GH treatment in the present study is most likely related to enhanced bone modeling associated with increased growth.

Osteocalcin and ICTP are filtered through the glomerular membrane and are higher in renal failure.[28] In the present study osteocalcin and ICTP were higher than those of healthy prepubertal children and related to creatinine clearance. PICP is cleared by the liver and is not expected to increase as a result of renal impairment.[33] However, at baseline, also PICP was higher than values of healthy prepubertal children. Therefore our data support increased bone turnover in children with CRF probably due to secondary hyperparathyroidism. In the no-GH-group, the increase of osteocalcin and IGFBP-3 may be related to the decrease of renal function.

The changes in body composition during GH treatment are in accordance with known lipolytic and anabolic effects of GH. Similar findings have been reported in GH deficient adults and children during GH treatment.[15,18,23]

In conclusion, mean BMD of children with CRF did not differ from normal. Height, bone turnover, and body composition of the children with CRF and growth retardation

changed during GH treatment corresponding with the changes observed in GH deficient children during GH treatment. In contrast to findings in GH-deficient children, no change was observed in BMD and vitamin D metabolism during GH treatment. In the no-GH-group BMD remained stable. Adequate treatment with alpha-calcidol might prevent osteopenia in patients with CRF.

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# Chapter 8

# Changes in bone mineral density, body composition and lipid metabolism during growth hormone treatment in children with growth hormone deficiency

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## Abstract

Adults with childhood onset growth hormone deficiency (GHD) have reduced bone mass, increased fat mass and disorders of lipid metabolism. The aim of the present study was to evaluate bone mineral density (BMD), bone metabolism, body composition and lipid metabolism in GHD children before and during 2 to 3 years of growth hormone treatment (GHRx). Forty children with GHD, mean age 7.9 years, participated in the study of bone metabolism and body composition and an additional group of 17 GHD children in the study of lipid metabolism. Lumbar spine BMD, total body BMD and body composition were measured with dual energy X-ray absorptiometry. Volumetric BMD (bone mineral apparent densiy, BMAD) was calculated to correct for bone size. BMD, BMAD, lean tissue mass, bone mineral content, fat mass, and percentage body fat were expressed as standard deviation scores (SDS) in comparison with normative data of the same population. Lumbar spine BMD and BMAD and total body BMD were all decreased at baseline. All BMD variables increased significantly during GHRx, lumbar spine BMD SDS already after 6 months of treatment. Lean tissue mass SDS increased continuously. Bone mineral content SDS started to increase after 6 months GHRx. Fat mass SDS decreased during the first 6 months of GHRx and remained stable thereafter. Biochemical parameters of bone formation and bone resorption did not differ from normal at baseline and increased during the first 6 months of GHRx. Serum 1,25 dihydroxyvitamin D increased continuously during GHRx, whereas PTH and serum calcium remained stable. Lipid profile was normal at baseline. Atherogenic index had decreased and apolipoprotein A1 had increased after three years of treatment.
In conclusion, children with GHD have decreased bone mass. BMD together with height and lean tissue mass increased during GHRx. GHRx had a beneficial effect on lipid metabolism.

## Introduction

Growth hormone (GH) is essential for normal growth during childhood and adolescence and influences bone mineralization and body composition in children as well as adults. In adults GH is known to affect lipid metabolism. GH deficiency (GHD) of childhood onset in adults and GHD in children is associated with reduced bone mineral density (BMD) and lean tissue mass and increased fat mass.[1-6] Studies in adults showed that these abnormalities change to normal after long-term treatment with human GH.[1,7] Few reports showed that BMD improved in GHD children during GH treatment (GHRx).[5,6] Bone mass accumulation during childhood determines the peak bone mass which is a major

determinant of osteoporosis later in life. Adults with GHD have an increased risk of cardiovascular disease because of disorders in lipid metabolism which improve during GHRx.[8,9] Reduction in triglycerides and low-density lipoprotein cholesterol and increase of high-density lipoprotein cholesterol have been observed in adults with GHD during therapy.[8-10] Reports of lipid metabolism during GHRx in children with GHD are contradictory.[11-13]

In the present study lumbar spine and total body BMD, lumbar spine BMD corrected for estimated bone volume, bone metabolism, body composition and lipid metabolism were evaluated in GHD children before and during 2 to 3 years of GHRx.

## Patients and Methods

*Patients*

Forty children (24 boys and 16 girls) participated in the study. The mean age at the start of treatment was 7.9 years (range 0.4 to 16.9). In two children younger than 4 years no BMD measurements were performed. In these young children it is difficult to lie still to perform the measurement. One of them became four years old after two years of treatment and then BMD measurements were performed.

All patients had decreased height velocity. The children had height SDS below 2 standard deviations (sd) compared withDutch reference values[14], except three patients. These children had a craniopharyngioma, low height velocity and height SDS below -1.

Eighteen children (14 boys and 4 girls, mean age 8.7 years) had classic GHD defined as two different GH provocation test peaks < 5 μg/l and insulin-like growth factor (IGF)-1 and IGF-binding protein 3 (IFGBP-3) below the mean of age- and sex-matched healthy controls.[15] The other 22 patients (10 boys and 12 girls, mean age 7.4 years) were categorized as nonclassic GHD. The diagnosis of 16 children was partial GHD, defined as GH provocation peaks between 5-10 μg/l and IGF1 and IFGBP-3 below the mean or GH provocation peaks between 10-15 μg/l and IGF1 and IFGBP-3 below 2 sd of normal. Five children formed a special group : two of them had GH provocation peaks more than 15 μg/l but IGF-I and IFGBP-3 below 2 sd, and two had GH peaks between 10-15 μg/l and IGF-I and IFGBP-3 that were 1.5 sd below normal. One girl had GH peaks between 10-15 μg/l but normal IGF-I. These five children had high IGF-I response during a test after stimulation with GH. One girl with intra-uterine growth retardation without catch-up growth had normal GH-peaks and IGF-I of -1 sd and a very low height SDS (-4.6 sd). Sixteen children had GHD of unknown origin. Eighteen had GHD of known origin : 8 had a malformation in the central nervous system, 2 had a syndrome with GHD (1 Prader-Willi and 1 Robinow), 7 had an intra-cerebral tumor (5 craniopharyngioma, 1 germinoma,

and 1 astrocytoma), 1 boy had received radiation therapy for a rhabdomyosarcoma of his left ear. Two children had Noonan syndrome and 4 were born after intra-uterine growth retardation.

Eight children had multiple pituitary hormone deficiencies and received hormonal replacement therapy. All patients had normal thyroid function before and during treatment. Thirty-two patients were prepubertal (14 with classic and 18 with nonclassic GHD), 5 had Tanner stage 2 (2 with classic and 3 with nonclassic GHD), 2 had Tanner stage 3 (1 with classic and 1 with nonclassic GHD) and 1 had Tanner stage 4 (with classic GHD).[16] Three children (1 with classic and 2 with nonclassic GHD) entered puberty during the study period.

In the above mentioned children and 17 other children (10 boys and 7 girls), mean age 7.9 years (sd 3.1), lipid metabolism was measured at baseline and during GH therapy. Of these 17 children, 6 patients had classic and 11 had nonclassic GHD. Eight had idiopathic GHD and 9 had GHD of known origin: 4 had a malformation in the central nervous system, 1 had an optic glioma, 1 had a pituitary microadenoma, 2 had hydrocephalus and 1 had neurofibromatosis. The child with the optic glioma had multiple pituitary deficiencies. Fifteen were prepubertal, 1 had Tanner stage 1 and 1 had Tanner stage 2. Lipid profile was evaluated at baseline in 55 children, after one year in 45 children, after two years in 33 children and after three years therapy in 16 children.

All patients were treated with daily subcutaneous injections of biosynthetic human GH 2 IU/$m^2$ body surface. The five children of the group of the IGF-I response test received 3 IU/$m^2$.

Informed consent was obtained from the parents of the patients.

*Methods*

Anthropometry, BMD and body composition measurements and assessment of biochemical bone parameters were performed at baseline and 6 months, one year and two years after onset of GHRx. BMD and body composition measurements at 6 months missed in one patient. Height was measured with a Harpenden stadiometer. Height was compared with age- and sex-matched reference values[14] and expressed as standard deviation scores (SDS). Body mass index was calculated as weight/(height)$^2$ (kg/$m^2$) and compared with age- and sex-matched reference values[17] and expressed as SDS.

BMD (g/$cm^2$) of the lumbar spine and total body was measured by dual energy X-ray absorptiometry (Lunar, DPXL/PED, Lunar Radiation Corporation, Madison, Wisconson, USA). The coefficient of variation has been reported as 1.04 % for lumbar spine and 0.64 % for total body.[18] The coefficient of variation (sd) for lumbar spine was 1.1 (0.2) % in our setting. Ancillary DXA-derived data were used to calculate lumbar spine volumetric BMD, bone mineral apparent density (BMAD), with the model BMAD = BMD x [4/ ($\pi$

x width)].[19] BMD and BMAD results were compared with our age- and sex-matched Dutch reference values (n=500, 4 to 20 years old)[20] and expressed as SDS. With the total body measurement by DXA the body composition was measured as lean tissue mass (g), fat mass (g) and bone mineral content (g). Total tissue mass is the sum of these three variables. Percentage body fat is given for total tissue mass. The coefficients of variation have been reported as 2.2 % for fat mass, 1.1 % for lean tissue mass and 0.6 % for bone mineral content.[18] Bone mineral content, lean tissue mass, fat mass and percentage body fat were compared to our age- and sex-matched Dutch reference values (4 to 20 years old) and expressed as SDS.[21]

Bone age was assessed yearly by one investigator (ME) using an X-ray of the left hand according to the Tanner-Whitehouse radius-ulna-short bones method.[22]

Blood samples were taken in the morning for the assessment of calcium, phosphate, alkaline phosphatase, 1,25 dihydroxyvitamin D, PTH, osteocalcin, the carboxy terminal propeptide of type I collagen (PICP), cross-linked telopeptide of collagen I (ICTP) and IGF-I. Osteocalcin and intact PTH were measured by radioimmunoassay (Incstar Corporation, Stillwater, MN, USA); 1,25 dihydroxyvitamin D by radioimmunoassay of Immuno Diagnostic Systems (Boldon, United Kingdom). PICP and ICTP was measured with a RIA kit (Orion Diagnostica, Espoo, Finland) with coefficients of variation of 4-6 % and 4-8 % respectively. Our own reference values for osteocalcin, PICP and ICTP (respectively n=25, n=82 and n=88) were used for prepubertal children. Reference values for the older children were derived from other studies that used the same assays.[23-25] For measurements of IGF-I (nmol/l) kits of Med-Genix Diagnostics, Fleurus, Belgium were used. IGF-I sex- and age-matched reference values were based on 600 samples of a healthy Dutch population.[15] In the first morning void of urine the ratio of hydroxyproline and creatinine (OHP/CR) and the ratio of calcium and creatinine (CA/CR) were evaluated.

Fasting blood samples were obtained yearly for the assessment of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), free fatty acids (FFA), apolipoprotein A1 (Apo-A1) and apolipoprotein B (Apo-B). Atherogenic index was calculated as the ratio of TC to HDL.

TC, TG, Apo-A1 and Apo-B were measured on DuPont de Nemours "Dimension" analyzer with reagents as provided by the manufacturer.[26] TC is liberated from all lipoprotein particles by detergents, the esterified fraction (about 70 % of TC) is hydrolyzed by the action of cholesterol esterase. TC is then oxidized by cholesterol oxidase which generates one mole of hydrogen peroxide for each mole of cholesterol. The peroxide generates a chromophore which is measured photometrically by a triple wavelength endpoint technique. The assay is calibrated by human serum samples with reference method based set points and is directly comparable with the standard method of Abell-

Kendall.[27] TG are converted to free glycerol by lipase and the glycerol is oxidized (by glycerol dehydrogenase) to dihydroxy-acetone under formation of NADH which is measured using a kinetic, bichromatic method. Pre-existing glycerol (usually below 0.1 mmol/l) is included in the result.[28]
HDL is analyzed after apo-B containing lipoprotein particles has been precipitated from the serum by heparin-MnCl2 solution.[29] LDL is calculated by the following Friedewald formula LDL = TC -(HDL + VLDL) where VLDL = TG * 0.45 mmol/l.[30]
Apo-B in the assay reacts with polyclonal anti-bodies against human Apo-B to form an immunoprecipitate; the reaction is enhanced by adding polyethyleneglycol. The endpoint turbidity is measured by photometry in a bichromatic procedure.[31] Apo-A1 is measured with polyclonal antibodies against human Apo-A1.[31] This reaction also is accelerated with polyethyleneglycol. FFA are measured by specific enzymatic esterification to Acyl-CoA and a follow-up reaction with acyl-CoA-oxidase to generate hydrogen peroxide. This hydrogen peroxide is then measured after color formation with a chromogen.[32] Ascorbate oxidase eliminates vitamin C interference. Dutch age-matched reference values were used for TC and HDL.[33] For the other lipids our own reference values of 59 healthy children between 2-10 years and available reference data[34] were used.

*Statistical analysis*
One sample t tests were performed to compare the mean SDS values with normal. Two sample t tests were used to compare variables with a normal distribution between two groups. We tested if the average within patient change differed from zero with one sample t-test. Pearson correlation coefficient was calculated to test the association between two variables with a normal distribution. Spearman's rank correlation coefficient was used in case of a non-normal distribution.

## Results

Table 1 shows the results of lumbar spine BMD and BMAD SDS, total body BMD SDS, height SDS, body mass index SDS and body composition variables at baseline and during GHRx. At baseline, lumbar spine BMD and BMAD SDS and total body BMD SDS were significantly lower than normal ($p<0.001$ for lumbar spine and total body BMD SDS and $p<0.02$ for BMAD SDS). Lumbar spine BMD and BMAD SDS and total body BMD SDS were below -2 in respectively 11, 3 and 5 patients. The results of boys did not differ significantly from these of girls. Lumbar spine BMD SDS increased already after 6 months GHRx, lumbar spine BMAD SDS after 2 years GHRx (Table 1).

**Table 1**

*Mean (sd) of different variables at baseline and during growth hormone treatment (GHRx). BMD=bone mineral density; SDS=standard deviation score; BMAD=bone mineral apparent density; BMI=body mass index. The mean within patient change from baseline was tested.*

| | baseline | 6 months GHRx | 1 year GHRx | 2 years GHRx |
|---|---|---|---|---|
| | n=38 | n=37 | n=33 | n=23 |
| lumbar spine BMD SDS | -1.62 (1.22) | -1.33 (1.17)[a] | -0.98 (1.11)[a] | -0.64 (0.87)[a] |
| lumbar spine BMAD SDS | -0.51 (1.21) | -0.50 (1.17) | -0.37 (1.06) | -0.19 (1.00)[a] |
| total body BMD SDS | -0.94 (1.20) | -1.35 (1.20)[c] | -1.02 (1.2) | -0.61 (1.16)[c] |
| bone mineral content SDS | -2.29 (1.11) | -2.36 (1.56) | -1.52 (1.29)[a] | -1.24 (0.99)[a] |
| lean tissue mass SDS | -2.72 (0.83) | -1.86 (0.87)[a] | -1.53 (0.79)[a] | -1.14 (0.54)[a] |
| fat mass SDS | -0.02 (1.76) | -0.59 (1.80)[b] | -0.31 (2.00)[b] | -0.59 (1.63) |
| % body fat SDS | 0.93 (2.11) | -0.39 (1.99)[a] | -0.10 (2.04)[a] | -0.45 (1.66)[b] |
| height SDS | -2.98 (0.76) | -2.32 (0.84)[a] | -1.86 (0.91)[a] | -1.63 (0.80)[a] |
| BMI SDS | 0.45 (2.40) | 0.24 (2.29) | 0.39 (2.42) | 0.37 (2.02) |

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.02$ compared to baseline

Total body BMD SDS decreased initially after 6 months GHRx and increased thereafter (Table 1). The observed changes remained if pubertal children were excluded. Mean lumbar spine and total body BMD SDS were still significantly lower than zero after 2 years GHRx ($p<0.01$ for lumbar spine and $p<0.02$ for total body); mean spine BMAD SDS did not differ significantly from zero. None of the patients had lumbar spine BMD or BMAD SDS below -2 after two years of treatment. Total body BMD SDS of 4 patients was below -2 at that time, three of them also had a low value at baseline. At baseline mean bone mineral content and lean tissue mass SDS were decreased ($p<0.001$) and percentage body fat increased ($p<0.02$). Bone mineral content SDS started to increase after 6 months GHRx. Lean tissue mass SDS and height SDS increased continuously during GHRx. Fat mass SDS and percentage body fat SDS decreased after 6 months GHRx and remained stable thereafter. After two years GHRx mean bone mineral content and lean tissue mass SDS were still significantly lower than normal (both $p<0.001$); mean fat mass SDS and percentage body fat SDS did not differ significantly from normal. The BMD variables, bone mineral content SDS, and lean tissue mass SDS did not differ significantly between patients with classic GHD and those with nonclassic GHD. Fat mass and percentage fat SDS were significantly higher in patients with classic GHD compared with the other patients (both $p<0.001$). The changes in lumbar spine BMD SDS, BMAD SDS,

total body BMD SDS, and body composition variables during GHRx was not significantly different between the classic and nonclassic GHD patients.

When BMD SDS was calculated for bone age ($SDS_{BA}$), mean lumbar spine BMD $SDS_{BA}$ at baseline was significantly decreased (mean -0.88, sd 1.11, $p<0.001$). Mean lumbar spine BMAD $SDS_{BA}$ and total body BMD $SDS_{BA}$ did not differ from normal. Because bone age was below 4 years in 12 children, no $SDS_{BA}$ could be calculated because we had no reference values of that age. During GH therapy, lumbar spine BMD $SDS_{BA}$ increased significantly; after 1 year the mean increase was 0.34 (sd 0.12) ($p<0.01$), from 1 to 2 years 0.30 (sd 0.42) ($p<0.01$). After 2 years GHRx, mean lumbar spine $SDS_{BA}$ was -0.54 (sd 0.79), significantly lower than zero ($p<0.02$). Total body BMD $SDS_{BA}$ decreased after 1 year (mean -0.45 (sd 0.70), $p<0.01$) and increased during the second year (mean 0.41 (sd 0.70), $p<0.05$). BMAD $SDS_{BA}$ did not change during the first year and increased during the second year (mean 0.22 (sd 0.35), $p<0.02$).

Table 2 shows the biochemical results at baseline and during GHRx. Mean osteocalcin, PICP and ICTP did not differ from normal at baseline. After 6 months GHRx, osteocalcin, PICP, ICTP, alkaline phosphatase, 1,25 dihydroxyvitamin D, IGF-I and OHP/CR had increased significantly. Osteocalcin and ICTP remained stable thereafter; PICP, alkaline phosphatase, phosphate and OHP/CR decreased significantly; 1,25 dihydroxyvitamin D and IGF-I continued to increase. PTH and CA/CR did not change during GHRx. The results were not different if pubertal children were excluded.

Table 3 shows the results of lipid metabolism at baseline and during GHRx. At baseline the mean levels of the lipid profile were normal. One child had TC above 6 mmol/l and three children had TG above 1.7 mmol/l. All patients had normal TC and TG after three years GHRx. Only Apo-A1 and atherogenic index changed significantly during GHRx; Apo-A1 had increased and atherogenic index had decreased after three years of GHRx.

At baseline, height SDS correlated with lumbar spine BMD and BMAD SDS ($r=0.43$, $p<0.01$ and $r=0.36$, $p<0.05$, respectively) and not with total body BMD SDS. Body mass index SDS related to all three BMD variables ($r= 0.51$ $p<0.01$ for lumbar spine BMD SDS, $r=0.52$ $p<0.001$ for lumbar spine BMAD SDS and $r=0.44$ $p<0.01$ for total body BMD SDS). Biochemical bone parameters and IGF-I SDS did not relate to lumbar spine BMD, BMAD SDS, total body BMD SDS or height SDS. IGF-I SDS correlated with lean tissue mass SDS ($r=0.35$, $p<0.05$) and not with bone mineral content, fat mass or percentage body fat SDS. Height SDS correlated with bone mineral content SDS and lean tissue mass SDS ($r=0.69$ respectively $r=0.62$, both $p<0.001$). The different lipids did not relate to fat mass or percentage body fat SDS or IGF-I SDS.

The change ($\Delta$) in height SDS between baseline and 2 years GHRx did not relate to $\Delta$ lumbar spine BMD or BMAD SDS or total body BMD SDS during the same period.

**Table 2**

*Mean (sd) of biochemical parameters at baseline and during growth hormone therapy (GHRx) in growth hormone deficient children. PICP=carboxy terminal propeptide of type I collagen; ICTP=cross-linked telopeptide of type I collagen; alkaline phosph.=alkaline phosphatase; PTH=parathyroid hormone, 1,25 OHD=1,25 dihydroxyvitamin D; IGF-I= insulin-like growth factor I; OHP/CR and Ca/CR =hydroxyprolin/creatinine and calcium/creatinine ratio (mmol/l per mmol/l) in first morning void of urine. The mean within patient change from baseline was tested.*

| | baseline | 6 months GHRx | 1 year GHRx | 2 years GHRx |
|---|---|---|---|---|
| | n=39 | n=38 | n=33 | n=21 |
| osteocalcin (µg/l) | 13.3 (4.7) | 20.8 (8.1)[a] | 20.7 (5.5)[a] | 16.7 (4.2) |
| PICP (µg/l) | 372 (240) | 582 (262)[a] | 458 (152) | 355 (149) |
| ICTP (µg/l) | 12.9 (4.6) | 17.4 (5.6)[a] | 18.1 (4.3)[a] | 16.8 (5.6) |
| alkaline phosph. (U/l) | 174.4 (51.7) | 245.7 (82.0)[a] | 255.6 (93.4)[a] | 226.0 (87.9)[c] |
| PTH (ng/l) | 18.9 (7.4) | 21.0 (7.5) | 24.4 (9.2) | 24.1 (11.3) |
| 1,25 OHD (pmol/l) | 96.2 (25.8) | 130.0 (49.3)[a] | 141.4 (46.5)[a] | 158.9 (41.1)[a] |
| IGF-I (nmol/l) | 17.0 (24.4) | 34.7 (25.4)[a] | 41.5 (29.3)[a] | 45.2 (31.1)[a] |
| Calcium (mmol/l) | 2.45 (0.09) | 2.45 (0.09) | 2.44 (0.08) | 2.44 (0.08) |
| Phosphate (mmol/l) | 1.41 (0.17) | 1.70 (0.21)[a] | 1.63 (0.18)[a] | 1.49 (0.15)[c] |
| Urine OHP/CR (mg/g) | 105 (77) | 133 (47)[b] | 151 (55)[b] | 118 (43) |
| Urine Ca/CR | 0.45 (0.46) | 0.46 (0.44) | 0.32 (0.25) | 0.39 (0.34) |

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.02$ compared to baseline

Δ height SDS related to Δ bone mineral content SDS ($r=0.41$, $p<0.02$ after 1 year and$r=0.44$, $p<0.05$ after 2 years GHRx). Only after 6 months GHRx, Δ 1,25 dihydroxyvitamin D had a significant correlation with Δ lumbar spine BMD and BMAD SDS ($r=0.34$, $p<0.05$ and $r=0.57$, $p<0.001$ respectively) and not with Δ total body BMD or bone mineral content SDS. Changes of the other biochemical bone parameters had no significant relation with Δ lumbar spine BMD or BMAD SDS or total body BMD or bone mineral content SDS. Only Δ ICTP after 6 months correlated with Δ height SDS during the same period ($r=0.48$, $p<0.01$ for all children, $r=0.40$, $p<0.05$ only for prepubertal children). Δ IGF-I SDS after 6 months or after 2 years did not relate to Δ height SDS. Δ 1,25 dihydroxyvitamin D between baseline and 2 years GHRx related to Δ IGF-I SDS ($r=0.52$, $p<0.05$). Δ IGF-I SDS after 6 months correlated with Δ lean tissue mass SDS ($r=0.45$, $<0.02$). Δ IGF-I SDS between baseline and 2 years correlated negatively with Δ percentage body fat ($r=-0.54$, $p<0.05$). Δ IGF-I SDS did not relate to Δ of any lipids or

**Table 3**

*Change in lipid profile during growth hormone treatment (GHRx). Mean (sd).*

*LDL=low-density lipoprotein cholesterol; HDL=high-density lipoprotein cholesterol; VLDL=very low density lipoprotein cholesterol; Apo=Apolipoprotein. Atherogenic index = total cholesterol to HDL ratio. The mean within patient change from baseline was tested.*

| | baseline | | 1 year GHRx | | 2 years GHRx | | 3 years GHRx | | reference values |
|---|---|---|---|---|---|---|---|---|---|
| | n | | n | | n | | n | | |
| Total cholesterol (mmol/l) | 55 | 4.41 (0.93) | 45 | 4.30 (1.26) | 33 | 4.17 (0.82) | 16 | 3.96 (0.85) | 3.2 - 6.0 |
| Triglycerides (mmol/l) | 55 | 0.98 (0.44) | 45 | 1.02 (0.41) | 33 | 0.98 (0.34) | 16 | 0.88 (0.23) | 0.2 - 1.7 |
| LDL (mmol/l) | 54 | 2.75 (0.87) | 43 | 2.63 (1.12) | 29 | 2.44 (0.61) | 14 | 2.46 (0.61) | 1.3 - 3.7 |
| HDL (mmol/l) | 55 | 1.21 (0.28) | 44 | 1.17 (0.29) | 33 | 1.26 (0.30) | 16 | 1.27 (0.27) | 0.9 - 1.6 |
| VLDL (mmol/l) | 53 | 0.44 (0.20) | 43 | 0.48 (0.20) | 24 | 0.45 (0.14) | 13 | 0.40 (0.09) | 0.1 - 0.8 |
| Free fatty acids (mmol/l) | 50 | 0.86 (0.47) | 45 | 0.73 (0.32) | 31 | 0.65 (0.25) | 15 | 0.61 (0.23) | 0.2 - 1.3 |
| Apo-A1 (g/l) | 54 | 1.35 (0.20) | 44 | 1.30 (0.18) | 30 | 1.39 (0.22) | 16 | 1.40 (0.16)[a] | 0.8 - 1.5 |
| Apo-B (g/l) | 54 | 0.85 (0.22) | 44 | 0.83 (0.29) | 30 | 0.83 (0.15) | 16 | 0.76 (0.17) | 0.6 - 1.1 |
| Atherogenic index | 55 | 3.85 (1.25) | 44 | 3.79 (1.53) | 33 | 3.41 (0.76) | 16 | 3.20 (0.72)[b] | |

[a] $p<0.05$, [b] $p<0.02$ compared to baseline

atherogenic index. After 2 years of treatment, IGF-I SDS related to lean tissue mass SDS ($r=0.52, p<0.05$) and not to the BMD variables or bone mineral content SDS.

## Discussion

During GHRx, lumbar spine BMD and BMAD and total body BMD together with height and lean tissue mass increased significantly in children with GHD. Biochemical parameters of bone formation and bone resorption increased indicating an enhanced bone turnover. GH has both direct and indirect actions on bone. In animal models, GH stimulates osteoblast number and function and the production of various bone matrix factors.[35] Paracrine activity of osteoblasts stimulates osteoclasts. Bone formation is enhanced preferentially to bone resorption during GH administration.[35] As in other tissues, part of the effects of GH are mediated through IGF-I. In childhood, GH affects both linear bone growth and the accumulation of BMD. In GHD children both are decreased as shown by low height SDS and BMD SDS. Some studies found a decreased osteocalcin and PICP[36,37] in GHD children, others did not find a difference with normals.[38,39] The wide range in normal values may be the reason that osteocalcin and PICP were in the normal range in the present study. Osteocalcin and PICP are related to linear growth velocity.[36,39,40] Increased values of all markers are found during periods of high growth velocity like the first years of life and puberty in healthy children.[40] During GHRx, the increase of both formation and resorption markers reflects growth, modeling, and remodeling of bone tissue. The increased bone turnover resulted in an improvement of BMD.

GH increases muscle mass, in agreement with our finding of an increase in lean tissue mass, and strength.[35] This may be associated with increased physical activity which may have a positive effect on BMD.

Lumbar spine BMD of adults with GHD decreases initially after three to six months GHRx and starts to increase after more than one year of treatment.[1,7] In the present study, lumbar spine BMD had increased and total body BMD decreased after 6 months treatment. BMD is an areal density and does not adjust for bone size completely. Total body bone mineral content remained stable during the first 6 months, so the initial decrease in total body BMD reflects a faster rate of bone expansion than mineral acquisition. Bone turnover in trabecular bone, present in lumbar spine, is higher than in cortical bone, 80 % of the total skeleton.[41] This may explain why lumbar spine BMD starts to increase earlier than total body BMD. During GHRx, patients had an increase of height SDS. After 2 years of treatment BMAD, corrected for estimated bone volume, had increased as well so one may conclude that finally true bone density improved during GHRx.

The changes in body composition found in the present study are in agreement with known

lipolytic and anabolic effects of GH. GHRx had a short-term lipolytic effect during the first 6 months of treatment while the anabolic effect continued. Similar results have been reported in other studies in adults and children with GHD and short stature children during GHRx.[1,42-44]

GH administration had a stimulatory effect on serum 1,25 dihydroxyvitamin D, whereas serum calcium and PTH remained unchanged. More studies found an increase in 1,25 dihydroxyvitamin D in adults[2] as well as in children.[4] Renal 1αhydroxylase activity is enhanced through IGF-I[45], which agrees with the correlation we observed between Δ in IGF-I SDS and Δ in 1,25 dihydroxyvitamin D. The synthesis of osteocalcin is induced by the action of 1,25 dihydroxyvitamin D.[46] Administration of 1,25 dihydroxyvitamin D to GHD children increased serum osteocalcin levels.[47] Part of the stimulatory action on osteoblastic activity of GH might be mediated by 1,25 dihydroxyvitamin D. This is supported by our finding of a correlation between the increase of 1,25 dihydroxyvitamin D and the increase in BMD.

In contrast to adults with GHD, the children in the present study had normal mean values of lipids at baseline. The difference in lipid profile between GHD adults and children may reflect the population trend for a rise in cholesterol and LDL with increasing age.[48] Studies in adults reported a decrease in TC, LDL and apo B after 2 to 12 months GH administration[8,9,49] and an increase of HDL.[10] The reported effects of GHD on serum lipids in children with GHD are inconsistent between studies. Some studies showed no changes in TC and HDL during 6 to 12 months of GHRx[12], whereas others found a decrease in TC[13] or an increase in HDL.[11] In the present study, the atherogenic index decreased, in agreement with a study of Kohno *et al.*[11] in prepubertal boys during 9 months of GHRx. In a study evaluating the efficacy of lipid profiles, the atherogenic index was the most efficient predictor of coronary heart disease in adults.[50] In healthy children no age-related change in TC was observed between 5 and 10 years of age, but TC decreased between 10 and 16 years in boys as well as in girls.[33] Mean HDL decreased slightly in boys and girls until the age of 17.[33] Therefore, the decrease in atherogenic index in the present study may be age-related. However, Apo-A1, the major apolipoprotein of HDL, increased as well so it seems that GHRx has a beneficial effect on lipid metabolism in children with GHD.

In conclusion, children with GHD had low BMD. After 2 years of GHRx lumbar spine BMD of all patients was within normal limits. Eighty-one percent of the patients had normal total body BMD at that time. The positive influence of GH on BMD might be mediated partly by the increase of 1,25 dihydroxyvitamin D. Fat mass decreased and lean tissue mass increased during treatment. In contrast to adults, children with GHD had a normal lipid profile. Atherogenic index improved during GHRx.

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# Chapter 9

# General discussion and future research

## Introduction

This thesis describes bone mineral density (BMD) and body composition of healthy children and of children with different diseases. Determinants of BMD and body composition were studied. In this chapter, first the results of BMD measurements of the patient groups are presented. Methodological constraints are mentioned. Discussion of the major determinants of BMD and body composition and of results of bone metabolism follows. Finally, clinical implications and suggestions for future research are given.

## Bone mineral density results of the patients groups

In chapter 2 determinants of BMD and body composition of healthy children and adolescents were described. The data obtained from these studies were used as reference values for the patients with diseases or treatment modalities which affect bone metabolism. Patients with the following conditions were studied :

- precocious and early puberty, before and during treatment with gonadotrophin-releasing hormone agonist (*Chapter 3*)
- chronic inflammatory bowel disease (*Chapter 4*)
- asthma and long-term treatment with inhaled corticosteroids (*Chapter 5*)
- acute lymphoblastic leukemia (*Chapter 6*)
- after renal transplantation (*Chapter 7.1*)
- chronic renal failure with and without growth hormone treatment (*Chapter 7.2*)
- growth hormone deficiency before and during growth hormone treatment (*Chapter 8*)

Our observations in various patient groups revealed a marked influence of the diseases or treatment modalities on BMD, as illustrated in Figure 1. The BMD results at baseline are presented of the longitudinal studies.

Mean lumbar spine BMD standard deviation score (SDS) of 34 children with early or precocious puberty was significantly higher than zero while total body BMD SDS did not significantly differ from zero (Figure 1). The higher spinal BMD at baseline is in agreement with other studies.[1-3]

Mean lumbar spine and total body BMD SDS were significantly lower than zero in children with inflammatory bowel disease (n=55), in young adults after renal transplantation (n=20) and in children with growth hormone deficiency (n=40) (Figure 1). Treatment with corticosteroids and nutritional status were important determinants of BMD in patients with inflammatory bowel disease. Children with Crohn's disease had lower BMD than children with ulcerative colitis. Corticosteroid treatment played a major part

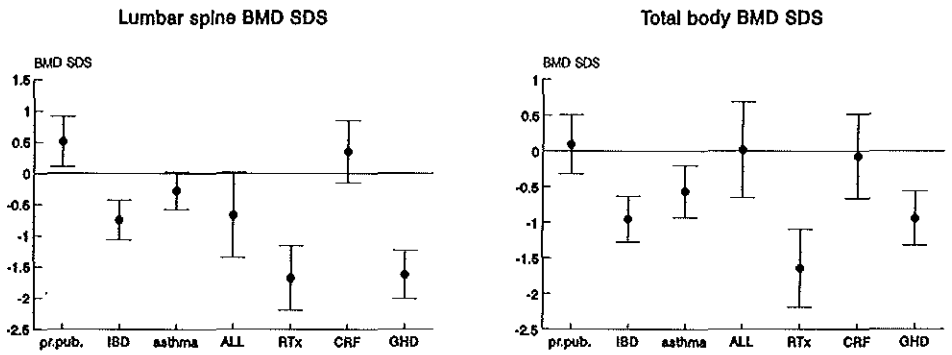


**Figure 1**

*Mean and 95 % confidence interval of lumbar spine bone mineral density (BMD) and total body BMD standard deviation score (SDS) of the patient groups described in this thesis. SDS is age- and sex-independent. pr.pub.= central precocious and early puberty (n=32), IBD = inflammatory bowel disease (n=55), asthma = asthma with long-term use of inhaled corticosteroids (n=40), ALL = acute lymphoblastic leukemia (n=14), RTx= after renal transplantation (n=20), CRF = chronic renal failure (n=31), GHD = growth hormone deficiency (n=38).*

in the development of post-renal transplantation osteopenia. Growth hormone influences bone metabolism and growth hormone deficiency is associated with reduced BMD.[4-7]
Mean total body BMD SDS was low in children with asthma and long-term use of inhaled corticosteroids (n=40). Duration of inhaled corticosteroid use correlated negatively with total body BMD SDS.
Lumbar spine BMD was decreased in three of fourteen patients with acute lymphoblastic leukemia at diagnosis. Leukemic infiltration of the bone marrow and marrow expansion have been mentioned as pathogenic factors of osteoporosis in acute lymphoblastic leukemia.[8]
Mean lumbar spine and total body BMD SDS did not significantly differ from normal in prepubertal children with chronic renal failure (n=36).

## Methodologic constraints

The studies of determinants of BMD and body composition in healthy children, BMD and bone turnover in asthmatic children, and BMD after renal transplantation are cross-sectional. In a cross-sectional study all the information is gathered simultaneously. This design allows to study associations between the disorder and the possible risk factor. The

determinant may either precede or follow the disease. Conclusions concerning causality can not be drawn. In our study on renal transplantation it is likely that corticosteroid treatment is a cause of the observed osteopenia. However, BMD measurements before renal transplantation are lacking and therefore the contribution of renal failure to the osteopenia can not be assessed. Prospective studies may shed more light on the causal pathway. The studies in children with precocious puberty, chronic inflammatory bowel disease, acute lymphoblastic leukemia, chronic renal failure, and growth hormone deficiency are longitudinal.

## Major determinants of BMD

*Glucocorticosteroids*

Steroid therapy is required for a number of diseases, such as inflammatory bowel disease, asthma, acute lymphoblastic leukemia, after a transplantation, rheumatoid arthritis, and chronic active hepatitis. Treatment with corticosteroids is the most frequent cause of osteoporosis in young patients. Glucocorticosteroids have a greater effect on trabecular bone than on cortical bone.[9,10] The bone loss is the results of a number of factors that adversely affect calcium homeostasis. Glucocorticosteroids inhibit gastrointestinal absorption and increase renal excretion of calcium. Negative calcium balance may increase parathyroid hormone (PTH) secretion. PTH increases the number of sites undergoing bone remodeling. Glucocorticosteroids inhibit osteoblastic bone formation at each site.[9]

We found a negative association between cumulative prednisone dose and BMD in children with chronic inflammatory bowel disease and in patients after renal transplantation. Some previous studies reported a relation between corticosteroids and BMD in patients with inflammatory bowel disease while others did not find this relationship.[11-13] Corticosteroids inhibit the inflammation. The inflammatory process and the malabsorption as a consequence of the inflammation may also negatively affect BMD. Therefore, the effect of corticosteroid treatment on BMD is complex in these patients.

In our study of young adults after renal transplantation, corticosteroid treatment was a major determinant of BMD. The patients had their first transplantation between 1977 and 1985. Since that time the immunosuppressive therapy has changed. A longitudinal study published in 1994 showed a decrease in spinal BMD 6 months after transplantation followed by an increase to 97 % of pretransplantation values at two years after transplantation in pediatric patients.[14] The incidence of osteoporosis of the patients who are transplanted at present may be less than that of patients transplanted ten to twenty years ago.

In asthmatic children, we reported a negative correlation correlation between the duration

of inhaled corticosteroid use and total body BMD. Although it is tempting to conclude that corticosteroids caused the decrease in BMD, asthma may have negatively influenced BMD. The duration of inhaled corticosteroid use may be a marker of the duration and severity of asthma. This is supported by finding a decrease in total body BMD and not in lumbar spine BMD, which contains a higher proportion trabecular bone.

In children with acute lymphoblastic leukemia total body BMD decreased during treatment with corticosteroids, methotrexate and other cytotoxic drugs. Corticosteroids primarily affect trabecular bone.[9] Therefore, the BMD decrease in these patients may not only be due to corticosteroids, but also to treatment with methotrexate. Methotrexate is known to affect bone metabolism.[15,16]

Corticosteroids are of undoubted therapeutic benefits but have side effects. Strategies to reduce side effects include alternate day administration and the use of osteotropic agents, which need further investigation (see further research).

*Puberty*

During puberty, BMD increases markedly in boys and girls. Bone mass accretion during puberty appears to be critical in the development of peak bone mass.[17] The Tanner stage in girls and weight in boys were the major independent determinants of BMD in our study of determinants of BMD in healthy children. Sex steroids have a great influence on bone mineralization.[18] Hypogonadism is associated with osteoporosis in both sexes. Testosterone insufficiency is a major risk factor for male osteoporosis.[19] The postmenopausal decline of estrogen levels cause the increased bone loss in women. Studies showed a directer effect of estrogens than of androgens in the mineralization of the skeleton.[20] Transgenic mice with "knock-out" of the estrogen receptor had significant undermineralization of the skeleton. In contrast, androgen-resistant mice (with knockout of the androgen receptor) had smaller bone sizes than male rats but normal BMD.[21] A man with estrogen resistance, caused by a disruptive mutation in the estrogen receptor gene, showed a severely undermineralized skeleton.[22] Adult patients with disruptive mutations in the androgen receptor, androgen insensitivity, also showed osteopenia.[23] The osteopenia in patients with androgen insensitivity may relate to defective androgen action, and inadequate estrogen replacement after gonadectomy (two of the five patients did not comply with the estrogen replacement therapy).[24] Androgens are converted to estrogens catalyzed by the enzyme aromatase. Aromatization of androgens is important for the mineralization of the skeleton.[21] Both inhibition of aromatization in the rat and aromatase deficiency in a man are associated with osteopenia.[21] Estrogens have an important role in the mineralization of the skeleton in males as well as in females. In pubertal boys, androgens together with growth hormone cause a large increase in height and bone sizes, lean tissue mass and weight. Androgens may have an indirect positive influence on BMD through their anabolic

function. Weight is known to increase BMD due to load on weight-bearing bones and was the major determinant of BMD in boys.

Late puberty is a risk factor of osteopenia.[24-26] Studies showed that late menarche and amenorrhoea in ballet dancers were related to reduced BMD and increased incidence of fractures.[24,25] Men who had experienced late puberty (onset after 15 years of age) had a significantly lower BMD of the radius and lumbar spine than men with puberty onset at normal age.[26] Late puberty is frequently observed in chronic diseases. We reported a delay of puberty in children with inflammatory bowel disease. The children with late puberty had lower BMD than the patients with a normal timing of puberty. Fifty-five percent of the patients who had received a renal transplantation had a late start of puberty. The delay in puberty may have contributed to the development of postrenal transplantation osteopenia.

Girls with early or precocious puberty had a high lumbar spine and normal total body BMD compared to controls. Increased estrogen and growth hormone levels associated with early puberty may have caused the high lumbar spine BMD. Changes in estrogen levels may affect trabecular bone faster than cortical bone, as has been shown in postmenopausal women.[27] The two boys who participated in the study had normal BMD. Precocious puberty is associated with premature and rapid skeletal maturation. Bone age is advanced more than one year beyond chronological age. If BMD SDS was calculated for bone age, mean lumbar spine and total body BMD appeared to be decreased. BMD SDS for bone age did not change significantly during two years of treatment with gonadotrophin-releasing hormone agonist. Long-term studies are needed to assess if these patients attain a normal peak bone mass. The patients of our study will be followed longitudinally.

*Nutritional status*

In healthy children, weight had a significant positive association with BMD. The association between body size and BMD is determined at least in part by skeletal responses to mechanical forces.[28] Children who are underweight are at risk of developing low BMD. Body mass index, lean tissue mass and fat mass were significantly decreased in children with inflammatory bowel disease. Body mass index had a positive relation with total body BMD in these patients and in the patients after renal transplantation.

Calcium intake correlated positively with BMD in boys in our study of determinants of BMD in healthy children. Studies showed that persons who consume greater quantities of calcium early in life have greater bone mass in adulthood.[29,30] An adequate calcium intake during childhood is important for optimal mineralization of the skeleton. About 14 % of the children with inflammatory bowel disease had a low calcium intake which may have contributed to the development of osteopenia.

*Height*

In most studies we found a correlation between height and BMD SDS. Disorders or treatment modalities may affect growth as well as BMD accretion, such as malnutrition, growth hormone deficiency and treatment with corticosteroids. Remarkably, the children with growth retardation due to chronic renal failure had normal BMD. Studies reported low BMD in adults with chronic renal insufficiency.[31-33] Growth retardation in chronic renal failure is not related to decreased growth hormone levels, but to diminished tissue response to growth hormone.[34] Adequate treatment with vitamin D may have prevented a decrease in BMD in these patients.

During growth hormone treatment BMD as well as height increased significantly in growth hormone deficient children. Growth hormone is responsible for longitudinal bone growth by stimulation of chondrocytes.[35] In animal models growth hormone treatment increased both the number and function of osteoblasts in vitro.[36] Many effects of growth hormone are mediated by insulin-like growth factor-I.[36] In growth hormone deficient patients 1,25-dihydroxyvitamin D increased during growth hormone treatment, in adults as well as in children.[5,37, Chapter 8] Growth hormone increases the production of 1,25-dihydroxyvitamin D by stimulating renal 1 α hydroxylase activity.[38,39] The most important action of vitamin D is the stimulation of calcium absorption in the gastro-intestinal tract. Furthermore, 1,25-dihydroxyvitamin D enhances the synthesis of osteocalcin by osteoblasts.[40] The increase of 1,25-dihydroxyvitamin D may be important for the stimulatory action on osteoblastic activity of growth hormone. The absence of a response in BMD during growth hormone treatment in children with chronic renal failure could be related to the lack of increase of 1,25-dihydroxyvitamin D.

*Physical activity*

Physical activity was positively correlated with BMD in boys in the reference study. The low variance in physical activity in girls may be the reason that no association was found between physical activity and BMD in girls. Other studies reported a positive association between BMD and physical activity in boys and girls.[41-43] Habitual loading leads to an increase of bone mass. Bone adapts to the mechanical demands that are placed to it.

In children with inflammatory bowel disease, the mean physical activity was not significantly lower than that of the children of the reference study. Physical activity did not relate to BMD. Other determinants of BMD may be more important in these patients. Prolonged bed rest, because of for example spinal cord injury, leads to rapid trabecular bone loss. The bone loss is associated with hypercalcaemia and is due to increased osteoclastic bone resorption and decreased osteoblastic bone formation.[44]

## Body composition

Socio-economic status related negatively to percentage body fat in girls in the study of determinants of body composition in healthy children. A study in the same population reported that overweight appeared to be more prevalent amongst children in poorer neighbourhoods.[45] Children with a high percentage body fat have an increased risk of cardiovascular diseases and diabetes.[46] Therefore, preventive programmes of obesity should be directed towards children and parents in less favourable social environments.

Physical activity had a positive association with lean tissue mass in boys, but did not influence fat mass. A study in children aged 6 to 17 years old reported a weak inverse relation between body fat and activity, suggesting that inactivity may not be a primary risk factor in childhood obesity.[47] In children aged 9 to 11 years, percentage body fat correlated positively with total and saturated fatty acids, and negatively with carbohydrate intake, also after adjustment for resting energy expenditure and physical activity.[48] These data suggested that diet composition may contribute to childhood obesity.

During puberty body composition changes markedly. Percentage body fat increased in consecutive Tanner stages in girls probably related to increased estrogen levels. In boys percentage body fat decreased from stage III to IV, which may be caused by increased growth hormone and androgens levels. Growth hormone increases significantly between stage III and IV in boys.[49] The higher growth hormone levels during puberty may explain the increase of lean tissue mass in boys and girls.

Children with precocious or early puberty had increased lean tissue mass and percentage body fat. During treatment with gonadotrophin-releasing hormone agonist lean tissue mass decreased and percentage body fat increased even further resembling the changes in body composition observed in patients with growth hormone deficiency. During treatment with gonadotrophin-releasing hormone agonist levels of estrogens and probably also levels of growth hormone decrease. Studies reported decreased growth hormone secretion and subnormal response to growth hormone stimulation tests during treatment with gonadotrophin-releasing hormone agonist in children with central precocious puberty.[50-52] The relative state of growth hormone deficiency during treatment may explain the changes in body composition. Oostdijk *et al.* reported that children with central precocious puberty with a markedly decreased height velocity during treatment may benefit of concomitant administration of growth hormone.[53]

In children with growth retardation due to asthma or chronic renal failure, lean tissue mass was decreased, but percentage body fat was normal. In case of malnutrition and growth retardation, which may occur in patients with chronic inflammatory bowel disease, both lean tissue mass and percentage body fat were decreased. Children with growth hormone deficiency had a decreased lean tissue mass but increased percentage body fat, as was

reported before in children as well as in adults with growth hormone deficiency.[5,54] Growth hormone is known to have anabolic and lipolytic effects. During growth hormone treatment lean tissue mass increased and fat mass decreased in the patients with growth hormone deficiency as well as in the patients with chronic renal failure.

## Bone metabolism

We assessed reference values of osteocalcin and carboxyterminal propeptide of type I collagen (PICP), both markers of osteoblastic activity, and cross-linked telopeptide of type I collagen (ICTP), a marker of bone resorption. The reference values were based on a limited number of samples of healthy prepubertal children. Our normal ranges were similar as reported in other studies.[55-57] Reference values of the pubertal children were taken from other studies which used the same assays. During puberty a considerable variation of PICP and osteocalcin concentrations between healthy subjects was reported.[58,59] A recent study showed that black females had lower bone turnover markers and higher BMD than white age-matched children.[60] Geographic and racial differences in levels of bone turnover markers may exist. The validity of our reference data would have been higher if the data were based on a higher number of samples of prepubertal and pubertal children of the Dutch population.

The wide range of the reference values may be the reason that osteocalcin and PICP levels of the children with growth hormone deficiency were within normal limits at baseline. Some studies reported low levels of osteocalcin and PICP in children with growth hormone deficiency, related to the decreased growth velocity and low BMD in these patients.[61,62] Others did not report a difference with normals, in agreement with our findings.[63,64]

In the longitudinal studies the change in bone turnover was evaluated. As described in the general introduction, markers of bone turnover may reflect bone modeling as well as bone remodeling. Both modeling and remodeling involve resorption and formation of bone. During growth hormone treatment biochemical parameters of bone turnover increased in growth hormone deficient children as well as in children with chronic renal failure. In growth hormone deficient children growth velocity and BMD increased during growth hormone treatment, therefore the increased bone turnover probably reflected bone modeling and increased bone formation in the remodeling process. In the children with chronic renal failure, growth hormone treatment increased growth velocity but did not influence BMD. In these children the increased bone turnover mainly reflected increased bone modeling. In children with precocious or early puberty bone turnover markers were higher those of prepubertal controls. Markers of bone metabolism are related to growt

velocity and increase maximally during midpuberty.[57,59] During treatment with gonadotrophin-releasing hormone agonist bone turnover decreased, probably reflecting a reduction in bone modeling.

No association was observed between markers of bone turnover and BMD. The biochemical parameters of bone turnover could not be used to predict BMD.

Changes in levels of the calcium-regulating hormones PTH and 1,25-dihydroxyvitamin D were observed in some patient groups. In children with acute lymphoblastic leukemia the decrease in total body BMD may be related to the observed increase in PTH during treatment. Vitamin D preparations suppress PTH secretion by both raising serum calcium and inhibiting PTH gene transcription.[65] Therefore, vitamin D treatment might be of benefit for these patients.

The increase of 1,25-dihydroxyvitamin D levels during growth hormone treatment in children with growth hormone deficiency has been discussed above.

Levels of 25-hydroxyvitamin D serve as an index of the adequacy of dietary vitamin D. Ten percent of the children with chronic inflammatory bowel disease had decreased 25-hydroxyvitamin D levels showing an inadequate intake or resorption of vitamin D. Vitamin D deficiency may have played a role in the development of osteopenia.

## Clinical implications

In children on long-term treatment with corticosteroids monitoring of BMD is required. Information about bone mass may permit improved patient management through, if possible, adjustments of dose, duration or way of administration (alternate day) of prednisone therapy, change to other drugs or additional treatment to prevent osteoporosis. BMD should be monitored in all children with inflammatory bowel disease with or without corticosteroid treatment. Also in children with other risk factors of osteoporosis like late puberty, malnutrition, and immobility assessment of BMD is required. Clinical assessment of bone metabolism starts with determination of calcium, phosphate and the calcium-regulating hormones PTH and 1,25-dihydroxyvitamin D. Hypocalcaemia stimulates secretion and synthesis of PTH, whereas hypercalcaemia suppresses both. The biochemical parameters of bone turnover as osteocalcin, PICP, ICTP and hydroxyproline, can not predict BMD. The markers may be used to provide insight in the pathogenesis of bone loss. A classification of bone disorders can be made in high, low, or dissociated bone turnover.[66] The effect of treatment on bone turnover can be evaluated. At present, the markers are only used in research.

## Future research

In this thesis risk factors of osteopenia were determined. Further studies are required concerning prevention and treatment of osteopenia in children. Treatment with calcium and calcitriol prevented corticosteroid-induced bone loss in the lumbar spine in a large group of adult patients.[67] However, about 25 % of the patients developed hypercalcaemia. In our study of BMD in children with chronic inflammatory bowel disease, patients with low BMD were treated with calcium 500 mg and vitamin D 400 IU per day. The change in BMD of these patients did not differ from the patients who were not treated with calcium and vitamin D. However, this study was not set up to evaluate the effect of calcium and vitamin D treatment on BMD. Vitamin D might be of benefit for the prevention of bone loss in these patients and in children with acute lymphoblastic leukemia. The best dose of calcium and vitamin D, the type of vitamin D, the conditions in which it is beneficial for prevention of bone loss in children need to be elucidated.

Although many studies have been performed to treat osteoporosis in adults, few studies have been done in children. Biphosphonates are commonly used for the treatment of involutional osteoporosis.[68] In children limited experience of the use of biphosphonates was gained in the treatment of juvenile osteoporosis[69] and osteogenesis imperfecta.[70] Biphosphonates are inhibitors of bone resorption. No dosage schedules for use in children are available. During treatment, bandlike metaphyseal sclerosis and epi- and apophyseal sclerosis developed in the growing skeleton.[71] The sclerosis seemed to be reversible (personal communication). Further studies need to be performed concerning treatment of osteoporosis with biphosphonates in children.

Growth hormone treatment had a positive effect on BMD in children with growth hormone deficiency. A few studies have been performed to evaluate the effect of growth hormone for treatment of corticosteroid induced osteoporosis.[72-74] Bone formation rates examined in bone biopsies increased in children with long-term corticosteroids during one year treatment with growth hormone.[29] Biochemical markers of bone turnover increased significantly during growth hormone administration in adults receiving chronic glucocorticoid treatment.[74] We treated an asthmatic boy with corticosteroid-induced osteopenia with daily subcutaneous growth hormone injections (4 IU/m$^2$) during two years. Lumbar spine BMD SDS increased from -3.5 to -2.3 and total body BMD SDS from -2.7 to -1.6. BMD as well as growth velocity increased during growth hormone treatment. Most children with osteopenia due to corticosteroid treatment are growth retarded. Growth hormone may have a beneficial effect both on BMD and growth. A study is planned to evaluate the effect of growth hormone treatment on BMD and growth in children with chronic inflammatory bowel disease and osteopenia. Patients with juvenile arthritis are commonly treated with corticosteroids. Studies reported low BMD in these children.[66,75]

Also in these children a study is planned to evaluate growth and BMD during growth hormone treatment.

In an ongoing study in adopted children with early puberty and combined treatment of gonadotrophin-releasing hormone and growth hormone growth will be evaluated. It would be interesting to compare BMD of these children with that of children treated only with gonadotrophin-releasing hormone.

In the general population, genetic factors strongly influence peak bone mass.[76,77] Twin studies showed genetic effects up to 80 % on BMD.[78,79] Forty-six to 62 % of the variance in BMD could be attributed to genetic factors in a study with parents and their children.[80] Genetic influences on BMD warrant exploration to identify persons at risk for osteoporosis. The complex biology of the skeleton, with many factors involved in skeletal growth and the variance of rate of bone loss in adults, make it extremely unlikely that there is a single gene for osteoporosis.[77] The inheritance of bone mass is probably under polygenic control, but the genes responsible are poorly defined.[81] An association was reported between vitamin D receptor gene alleles and BMD in postmenopausal women.[82,83] Furthermore, vitamin D receptor gene polymorphisms may predict bone loss in the elderly.[84] A study performed in children, aged 8-21 years, showed no relation between vitamin D genotype and forearm BMD gain or in BMD assessed at the forearm, spine, hip, and whole body.[85] Polymorphism in the estrogen receptor gene and collagen I gene may also be associated with BMD.[77,81] Bone mass of adults is the result of many environmental influences acting on the genetic potential for peak bone mass. Genetic studies on BMD may best be performed in children or young adults to minimize environmental influences.[77]

Recently, equipment has been developed to assess bone status by ultrasound. The instrument measures the speed of propagation of ultrasound waves (SOS, meters per second) along a fixed longitudinal distance of the cortical layer at the tibial shaft.[86] The technique is believed to reflect both qualitative and quantitative aspects of the bone.[87] In adults, cortical velocity seemed particularly well suited for assessing appendicular fracture risk.[87] Further studies are required to determine the clinical relevance of this technique, in adults as well as in children.

Reference values of BMD and body composition of Dutch children enables evaluation of BMD and body composition of patients with disorders which may affect bone metabolism or body composition. Assessment of determinants of BMD during childhood and adolescence, as performed in this thesis, may give direction to preventive measures or possible treatments of osteoporosis. Ongoing, long-term follow up of the patients till late adolescence is required to evaluate their peak bone mass, a major determinant of osteoporosis later in life. Very long-term longitudinal studies may assess the impact of low BMD during childhood and adolescence on the risk of fractures in adulthood.

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## Summary

In this thesis bone mineral density (BMD) and body composition were studied in healthy children and in children with diseases or treatments which may affect bone metabolism. Determinants of BMD and body composition were evaluated. *Chapter 1* introduces the subject. During childhood bone mass increases with age till the peak bone mass is attained. The peak bone mass is a major determinant of bone mass later in life. Children with a low BMD have a higher risk of fractures and may have a higher risk of osteoporosis in adult life. Preventive measures for osteoporosis later in life are focused on increasing peak BMD. In the present studies lumbar spine BMD, total body BMD and body composition were assessed with dual energy X-ray absorptiometry (DXA). Lumbar spine consists for the greater part of trabecular bone, the bone of the total body consists mainly of cortical bone. Knowledge of body composition is important in metabolic and nutritional studies. Diseases or drugs which affect bone metabolism may also influence body composition. Body composition by DXA assesses fat mass, lean tissue mass and bone mineral content. Biochemical markers of bone formation and bone resorption may provide insight in the pathogenesis of osteoporotic disorders. In growing children, modeling of new bone and remodeling of existing mineralized tissue are each ongoing processes. Both involve bone formation and bone resorption. The biochemical markers are not specific for either modelling or remodelling.

In *Chapter 2.1* the association of height, weight, pubertal stage, calcium intake and physical activity with BMD was evaluated in 500 healthy children and adolescents (205 boys and 295 girls), aged 4 to 20 years. Lumbar spine BMD, total body BMD and spinal volumetric BMD increased with age. After adjustment for age, height had a significant positive association with lumbar spine and total body BMD in boys and with lumbar spine BMD in girls. Weight correlated with all BMD variables in boys and girls, after adjustment for age. During puberty the age-dependent increment was higher. After adjustment for age, the Tanner stage was significantly associated with all BMD variables in girls and with spinal BMD in boys. Late puberty and amenorrhea are risk factors of low BMD in girls. In boys positive correlations were found between BMD and both calcium intake and physical activity, after adjustment for age. An adequate calcium intake during childhood appears to be important for optimal mineralization of the skeleton. Children who are underweight and inactive are at risk of developing low BMD. The major independent determinant of BMD was the Tanner stage in girls and weight in boys.

In *Chapter 2.2* determinants of body composition were evaluated in a cross-sectional study of 403 healthy Dutch children and adolescents. In 85 subjects the results of bio-

electrical impedance analysis (BIA) were compared with DXA. Fat mass, lean tissue mass and bone mineral content increased with age in boys and girls. Percentage body fat did not change with age in boys. Girls had higher percentage body fat than boys at all ages, and in them the percentage increased with age. From the age of 14 years boys had higher lean tissue mass and bone mineral content than girls. Tanner stage had a significant relation with body composition in both sexes. Percentage body fat was lower in boys in stage 4 than in stage 3, which could be related to the increases of growth hormone and androgens levels during puberty. In girls, percentage body fat was higher in consecutive Tanner stages. After adjustment for age, Tanner stage was significantly positively related to lean tissue mass and bone mineral content in boys and girls and to percentage body fat and fat mass in girls. A higher rank of profession of the parents and a higher education of the father related to lower percentage body fat or fat mass in their daughters, adjusted for age. High physical activity was related to higher lean tissue mass but was not related to fat mass in boys. A high correlation and a small difference was found between lean body mass by BIA and lean tissue mass by DXA.

*Chapter 3* reports BMD, bone metabolism and body composition of children (32 girls and 2 boys) with central precocious and early puberty before and during treatment with gonadotrophin-releasing hormone agonist. This treatment is supplied to arrest pubertal development. Patients were studied at baseline and during treatment for two years. Mean lumbar spine BMD standard deviation score was significantly higher than normal at baseline and did not differ from normal after two years of treatment. Volumetric spinal BMD and total body BMD were not different from normal before treatment and remained stable during treatment. During therapy, fat mass and percentage body fat increased, whereas lean tissue mass decreased. The changes in body composition resembled those seen in patients with growth hormone deficiency. Mean lumbar spine and total body BMD and volumetric BMD for bone age were all lower than normal at baseline and also after two years treatment. Biochemical bone parameters were significantly higher than prepubertal values at baseline and decreased during treatment. Long-term follow-up till peak bone mass is needed to evaluate BMD and body composition after cessation of treatment.

*Chapter 4* describes BMD, nutritional status and determinants of BMD in 55 children (34 boys and 21 girls) with inflammatory bowel disease. Twenty-two children suffered from Crohn's disease and 33 children from ulcerative colitis. Yearly measurements during two years were performed in 21 patients. Lumbar spine BMD and total body BMD were lower than normal. Also height and body mass index were decreased. The decrease in BMD standard deviation score could not only be explained by delay in bone maturation. A high cumulative dose of prednisone related to a low lumbar spine

BMD. Good nutritional status related to higher BMD. Patients with Crohn's disease had lower lumbar spine and total body BMD than patients with ulcerative colitis, even after adjustment for cumulative dose of prednisone. In the longitudinal study, the cumulative dose of prednisone between the measurements had a negative correlation with the change in lumbar spine and total body BMD. Conclusions of this study are : Children with inflammatory bowel disease are at risk to develop osteoporosis. Patients with Crohn's disease have a higher risk than patients with ulcerative colitis. Corticosteroid therapy and nutritional status are important determinants of BMD in these patients.

*Chapter 5* shows BMD, bone metabolism, height, and body composition of 40 prepubertal children (19 girls, 21 boys) with asthma, treated with a moderate to high dose of inhaled corticosteroids during 3 to 8 years. BMD results were compared with 148 prepubertal healthy children of the same population. The asthmatic children had a decreased height, lean tissue mass and fat mass and a delay of bone maturation, indicating growth retardation. Asthma had a significant negative relation with total body BMD in a multiple regression model with adjustment for age, sex, height and weight. Duration of inhaled corticosteroids use correlated negatively with total body BMD if it was added to the model. Cumulative dose of inhaled corticosteroids had no significant relation with total body BMD. The period of inhaled corticosteroids use is a marker of duration and severity of asthma. Therefore, the reduction of total body BMD may be due to the chronic illness rather than to the effect of use of inhaled corticosteroids. Asthma had no significant relation with lumbar spine BMD. If age of the asthmatic children was replaced by their bone age in the model no significant correlation was found between asthma and total body or lumbar spine BMD. The biochemical parameters of bone metabolism were within normal limits.

*Chapter 6* reports lumbar spine and total body BMD and bone metabolism of children with acute lymphoblastic leukaemia at diagnosis, during treatment with chemotherapy and one year after completion of treatment. Thirty-two children (21 boys and 11 girls) participated in the study. Fourteen children started the study at diagnosis and 18 during or after the treatment period. Three of 14 children had low lumbar spine BMD at diagnosis, which may have been caused by leukaemic infiltration of the bone marrow and marrow expansion. All children had normal total body BMD at diagnosis. Markers of bone turnover were depressed. Total body BMD decreased significantly during the first year of treatment, suggesting a negative effect of chemotherapy on cortical bone. The mean decrease was 1.1 standard deviation. Lumbar spine BMD did not change during treatment. Parameters of bone turnover increased to normal during the treatment period. Parathyroid hormone had increased significantly after 1 year. Parathyroid hormone was above normal levels in two patients. Mineral homeostasis was disturbed in some patients during treatment. Three of 7 patients had low total body BMD 1 year

after completion of treatment. All patients had normal lumbar spine BMD and normal biochemical results at that time. Further studies are needed to evaluate the long-term effect of leukaemia and its treatment on BMD.

In *Chapter 7.1* a cross-sectional study assessed BMD of 20 young adult patients (13 mean and 7 women) who received a renal transplantation in childhood. The time since the first transplantation varied from 9 to 17 years (mean 14 years). The majority of the patients had moderate to severe osteopenia. Both lumbar spine as total body BMD were affected. The mean standard deviation score of lumbar spine was -1.7 and of total body -1.6. A high cumulative dose of prednisone related to a low lumbar spine and total body BMD. Final height had a positive association with BMD. Corticosteroid treatment played a major part in the development of osteopenia in these patients.

*Chapter 7.2* describes BMD, bone metabolism and body composition of 36 prepubertal children with chronic renal failure. Seventeen children with growth retardation were treated with growth hormone during one year, after an observation period of six months. They were compared with a group of children with chronic renal failure who were not treated with growth hormone, most of them had no growth retardation. In both groups lumbar spine BMD, total body BMD, biochemical markers of bone metabolism, and body composition were assessed every six months. At baseline, mean lumbar spine and total body BMD were not different from normal. After one year of growth hormone treatment, lumbar spine BMD and total body BMD had not changed significantly. Height and biochemical markers of bone formation and bone resorption increased during treatment, levels of 1,25 dihydroxyvitamin D remained stable. Lean tissue mass increased and fat mass decreased during growth hormone therapy. BMD and height standard deviation score, the markers of bone turnover which are independent of renal function, and body composition remained stable in the patients not treated with growth hormone. In conclusion, BMD of children with renal failure did not differ from healthy controls. BMD remained stable, in the children treated with growth hormone as well as in the children not treated with growth hormone. Adequate treatment with alpha-calcidol might have prevented osteopenia in these patients.

In *Chapter 8* bone mineral density, bone metabolism, body composition and lipid metabolism in growth hormone deficient children were evaluated before and during 2 to 3 years of growth hormone treatment. Forty children with growth hormone deficiency, mean age 7.9 years, participated in the study of BMD, bone metabolism and body composition. An additional group of 17 growth hormone deficient children, mean age 7.9 years, joined the study of lipid metabolism. Lumbar spine BMD and volumetric BMD and total body BMD were all decreased at baseline. All BMD variables increased significantly during growth hormone treatment, lumbar spine BMD already after 6 months of treatment. Height and lean tissue mass increased continuously. Fat mass

decreased during the first 6 months of treatment and remained stable thereafter. Biochemical parameters of bone formation and bone resorption did not differ from normal at baseline and increased during the first 6 months of treatment. Serum 1,25-dihydroxyvitamin D increased continuously during growth hormone treatment, and the increase correlated with the change of height and insulin-like growth factor I standard deviation scores. After three years of treatment, the atherogenic index had decreased and Apolipoprotein A1 had increased, indicating a beneficial effect of growth hormone on lipid metabolism in children with growth hormone deficiency.
In *Chapter 9*, the major determinants of BMD during childhood reported in the studies are discussed. Results of body composition and bone metabolism are evaluated and clinical implications are mentioned. Recommendations are made and suggestions for future research are given concerning prevention and treatment of low BMD in children.

## Samenvatting

In dit proefschrift is onderzoek beschreven naar botdichtheid en lichaamssamenstelling bij gezonde kinderen en adolescenten en bij kinderen met ziekten of medicatie die het botmetabolisme kunnen beïnvloeden. Determinanten van botdichtheid en lichaamssamenstelling zijn geëvalueerd. *Hoofdstuk 1* geeft een inleiding. Tijdens de kinderleeftijd neemt de botmassa toe totdat de piekbotmassa wordt bereikt. De piekbotmassa is een belangrijke determinant van de botdichtheid op oudere leeftijd. Kinderen met een lage botdichtheid hebben een verhoogd risico op fracturen en mogelijk op osteoporose bij het ouder worden. Maatregelen ter preventie van osteoporose zijn gericht op het verhogen van de piekbotdichtheid. In het onderzoek beschreven in dit proefschrift zijn de botdichtheid van de lumbale wervelkolom en van het hele skelet en de lichaamssamenstelling gemeten met 'dual energy x-ray absorptiometry' (DXA). De lumbale wervelkolom bestaat overwegend uit trabeculair bot. Het hele skelet bestaat voor ongeveer 80 procent uit corticaal bot. Kennis van de lichaamssamenstelling is belangrijk bij metabole studies en voedingsonderzoek. Ziekten en medicatie die effect hebben op het botmetabolisme kunnen tevens de lichaamssamenstelling beïnvloeden. De lichaamssamenstelling wordt gemeten met DXA als vetmassa, vetvrij weefsel en botmassa. Biochemische parameters van botformatie en botresorptie kunnen inzicht verschaffen in de pathogenese van osteoporotische aandoeningen. In groeiende kinderen vinden zowel opbouw van nieuw bot als omzetting van bestaand gemineraliseerd weefsel plaats. Bij beide processen treden botformatie en botresorptie op. De biochemische botparameters zijn niet specifiek voor hetzij botopbouw hetzij botombouw.
*Hoofdstuk 2.1* beschrijft het onderzoek naar de associatie van botdichtheid met lengte,

gewicht, puberteitsstadium volgens Tanner, calciuminname in de voeding en lichamelijke activiteit bij 500 gezonde kinderen en adolescenten (205 jongens en 295 meisjes), in de leeftijd van 4 tot 20 jaar. De botdichtheid van de lumbale wervelkolom en het hele skelet en de volumetrische botdichtheid van de wervelkolom namen toe met de leeftijd. Tijdens de puberteit trad een versnelde toename op. Gecorrigeerd voor leeftijd had lengte had een positieve associatie met de botdichtheid van de lumbale wervelkolom en het hele skelet bij jongens en met de botdichtheid van de lumbale wervelkolom bij meisjes. Gewicht correleerde met alle voor leeftijd gecorrigeerde botdichtheid variabelen bij jongens en meisjes. Het puberteitsstadium had een significante correlatie met alle voor leeftijd gecorrigeerde botdichtheid variabelen bij meisjes en met de botdichtheid van de lumbale wervelkolom bij jongens. Late puberteit en amenorroe zijn risicofactoren voor een lage botdichtheid bij meisjes. Bij jongens correleerde de voor leeftijd gecorrigeerde botdichtheid positief met zowel calciuminname als lichamelijke activiteit. Voldoende calciuminname in de voeding is belangrijk voor optimale mineralisatie van het skelet tijdens de kinderleeftijd. Ondervoede en niet actieve kinderen hebben een verhoogd risico een lage botdichtheid te ontwikkelen. De belangrijkste onafhankelijke determinant van de botdichtheid was het puberteitsstadium bij meisjes en het gewicht bij jongens.

In *Hoofdstuk 2.2* worden determinanten van lichaamssamenstelling geëvalueerd in een transversale studie bij 403 gezonde Nederlandse kinderen en adolescenten. Bij 85 proefpersonen werden de resultaten van de 'bio-electrical impedance' analyse (BIA) vergeleken met de DXA. Vetmassa, vetvrij weefsel en botmassa namen toe met de leeftijd bij jongens en meisjes. Bij jongens veranderde het percentage lichaamsvet niet met de leeftijd. Meisjes hadden op elke leeftijd een hoger percentage lichaamsvet dan jongens en bij hen nam dit percentage toe met de leeftijd. Vanaf de leeftijd van 14 jaar hadden jongens meer vetvrij weefsel en een hogere botmassa dan meisjes. Het puberteitsstadium was significant gecorreleerd met lichaamssamenstelling bij beide geslachten. Bij jongens nam het percentage lichaamsvet af vanaf puberteitsstadium 3. Dit kan samenhangen met de toename van groeihormoon en androgeenspiegels tijdens de puberteit bij jongens. Bij meisjes nam het percentage lichaamsvet toe met de opeenvolgende puberteitsstadia. Na correctie voor leeftijd had het puberteitsstadium een positieve correlatie met de massa vetvrij weefsel en botmassa bij jongens en meisjes en met het percentage vet en de vetmassa bij meisjes. Een hogere beroepsstatus van de ouders of een hoger opleidingsniveau van de vader correleerde met een lager percentage lichaamsvet of vetmassa bij meisjes. Bij jongens was er een positieve relatie tussen de mate van lichamelijke activiteit en de hoeveelheid vetvrij weefsel maar niet met de vetmassa. Een hoge correlatie en een klein verschil werd gevonden tussen vetvrije massa gemeten met BIA en massa vetvrij weefsel gemeten met DXA.

*Hoofdstuk 3* beschrijft de botdichtheid, botmetabolisme en lichaamssamenstelling van kinderen (32 meisjes en 2 jongens) met centrale pubertas praecox en vroege puberteit voor en tijdens de behandeling met het gonadotropine 'releasing' hormoon agonist. Deze behandeling wordt gegeven om de puberteitsontwikkeling tegen te houden. De kinderen werden gezien bij het begin van de behandeling en gedurende twee jaar tijdens de behandeling. De gemiddelde botdichtheid van de lumbale wervelkolom was significant hoger dan normaal vóór de behandeling en verschilde niet meer van normaal na twee jaar behandeling. De volumetrische botdichtheid van de wervels en de botdichtheid van het hele skelet verschilden niet van normaal zowel vóór als na twee jaar behandeling. Tijdens de behandeling namen de vetmassa en het percentage lichaamsvet toe, terwijl het vetvrije weefsel afnam. De verandering in lichaamssamenstelling kwam overeen met die van patiënten met groeihormoondeficiëntie. De gemiddelde botdichtheid beoordeeld voor de skeletleeftijd was lager dan normaal zowel vóór als na twee jaar behandeling. Biochemische botparameters waren aanvankelijk significant hoger dan prepubertaire waarden en namen af tijdens de behandeling. Langdurig onderzoek van de botdichtheid en lichaamssamenstelling na staken van de behandeling is geïndiceerd.

*Hoofdstuk 4* beschrijft de botdichtheid, voedingsstatus en determinanten van botdichtheid bij 55 kinderen (34 jongens en 21 meisjes) met chronische inflammatoire darmziekte. Tweeëntwintig kinderen leden aan de ziekte van Crohn, en 33 aan colitis ulcerosa. Jaarlijkse metingen gedurende twee jaar werden verricht bij 21 patiënten. De botdichtheid van de lumbale wervelkolom en het hele skelet waren lager dan normaal. Ook de lengte en de Quetelet index waren verminderd. De lage botdichtheid kon niet verklaard worden door een achterstand in botmaturatie. Een hoge cumulatieve dosis prednison relateerde met een lage botdichtheid van de lumbale wervelkolom. Een goede voedingsstatus relateerde met een hoge botdichtheid. Patiënten met de ziekte van Crohn hadden een lagere botdichtheid van de lumbale wervelkolom en het hele skelet dan kinderen met colitis ulcerosa, zelfs na correctie voor de cumulatieve prednison dosis. In het longitudinale onderzoek bleek dat ook het prednison gebruik tussen de metingen een negatieve effect had op de botdichtheid. De conclusies van deze studie zijn als volgt: Kinderen met chronische inflammatoire darmziekte lopen risico op het ontwikkelen van osteoporose. Voor patiënten met de ziekte van Crohn is dit risico groter dan voor patiënten met colitis ulcerosa. Behandeling met corticosteroïden en de voedingsstatus zijn belangrijke determinanten van de botdichtheid bij deze patiënten.

*Hoofdstuk 5* beschrijft de botdichtheid, het botmetabolisme, de lengte en de lichaamssamenstelling van 40 prepubertaire kinderen (19 meisjes en 21 jongens) met astma die continu behandeld werden met een matige tot hoge dosis inhalatiecorticosteroïden gedurende drie tot acht jaar. De uitslagen van de botdichtheid van deze kinderen

werden vergeleken met 148 prepubertaire gezonde kinderen uit dezelfde populatie. De kinderen met astma hadden een kleine lengte, relatief weinig vetvrij weefsel en vet en een vertraagde skeletrijping, passend bij een groeiachterstand. Astma bleek negatief gecorreleerd te zijn met de botdichtheid van het hele skelet in een multipel regressie model met correctie voor leeftijd, geslacht, lengte en gewicht. De duur van het gebruik van inhalatiecorticosteroïden correleerde negatief met de botdichtheid van het hele skelet als deze parameter aan het model werd toegevoegd. De cumulatieve dosis van inhalatiecorticosteroïden had echter geen verband met de botdichtheid van het hele skelet. De duur van het gebruik van inhalatiecorticosteroïden houdt verband met de duur en de ernst van de ziekte. De gevonden lage botdichtheid zou veroorzaakt kunnen zijn door de chronische ziekte en niet zozeer door het gebruik van inhalatiecorticosteroïden. Astma had geen significante correlatie met de botdichtheid van de lumbale wervelkolom. Als de leeftijd van de kinderen met astma vervangen werd door de skeletleeftijd, verdween de relatie gevonden tussen astma en de botdichtheid. De biochemische botparameters waren binnen de normale grenzen.

*Hoofdstuk 6* beschrijft de botdichtheid van de lumbale wervelkolom en het hele skelet en het botmetabolisme bij kinderen met acute lymfatische leukemie bij diagnose, tijdens behandeling met chemotherapie en één jaar na voltooiing van de behandeling. Tweeëndertig kinderen (21 jongens en 11 meisjes) namen deel aan het onderzoek. Veertien kinderen werden geïncludeerd ten tijde van de diagnose en 18 tijdens of na de behandeling. Drie van de 14 kinderen hadden een lage botdichtheid van de lumbale wervelkolom bij diagnose, wat veroorzaakt kan zijn door leukemische infiltratie van het beenmerg en beenmerg expansie. Alle kinderen hadden een normale botdichtheid van het hele skelet bij diagnose. Parameters van botomzetting waren verlaagd. De botdichtheid van het hele skelet nam significant af in het eerste jaar van de behandeling. Dit suggereert een negatief effect van de chemotherapie op corticaal bot. De gemiddelde afname was 1.1 standaard deviatie. De botdichtheid van de lumbale wervelkolom veranderde niet tijdens de behandeling, terwijl de parameters van botomzetting toenamen tot normale waarden. Het parathyroïdhormoon was significant toegenomen na één jaar. Bij twee patiënten was het parathyroïdhormoon boven de normale waarden. De calciumstofwisseling was gestoord in enkele patiënten tijdens de behandeling. Drie van de zeven patiënten hadden een lage botdichtheid van het hele skelet één jaar na het einde van de therapie. Alle patiënten hadden op dat tijdstip een normale botdichtheid van de lumbale wervelkolom en normale biochemische uitslagen. Onderzoek is nodig om het langdurig effect van leukemie en de behandeling hiervan op de botdichtheid te evalueren.

In *Hoofdstuk 7.1* wordt in een transversale studie de botdichtheid van 20 jong volwassen patiënten (13 mannen en 7 vrouwen) die een niertransplantatie op de kinderleeftijd

hadden ondergaan geëvalueerd. De tijd sinds de eerste transplantatie varieerde van 9 tot 17 jaar (gemiddeld 14 jaar). De meerderheid van de patiënten had matige tot ernstige osteopenie. Zowel de botdichtheid van de lumbale wervelkolom als de botdichtheid van het hele skelet waren verminderd. De gemiddelde standaard deviatie score van de lumbale wervelkolom was -1.7 en van het hele skelet -1.6. Een hoge cumulatieve dosis prednison correleerde met lage botdichtheid van de lumbale wervelkolom en het hele skelet. Eindlengte had een positieve associatie met de botdichtheid. Behandeling met corticosteroïden speelt waarschijnlijk een belangrijke rol bij het ontstaan van de osteopenie bij deze patiënten.

*Hoofdstuk 7.2* beschrijft de botdichtheid, het botmetabolisme en de lichaamssamenstelling van 36 prepubertaire kinderen (27 jongens en 9 meisjes) met chronische nierinsufficiëntie. Zeventien kinderen met groeiretardatie werden behandeld met groeihormoon gedurende één jaar na een observatie periode van 6 maanden. Ze werden vergeleken met een groep kinderen met chronische nierinsufficiëntie die niet behandeld werden met groeihormoon en waarvan het merendeel normaal groeide. Bij beide groepen werd de botdichtheid van de lumbale wervelkolom en het hele skelet, biochemische parameters van botomzetting en de lichaamssamenstelling elke 6 maanden gemeten. De gemiddelde botdichtheid van de lumbale wervelkolom en het hele skelet verschilde niet van normaal bij de eerste meting. Beide botdichtheid variabelen waren niet veranderd na één jaar behandeling met groeihormoon. De lengte en de biochemische parameters van botomzetting namen wel significant toe tijdens de behandeling. De spiegel van het 1,25 dihydroxyvitamine D bleef gelijk. De hoeveelheid vetvrij weefsel nam toe en de vetmassa nam af tijdens de therapie. De botdichtheid en lengte standaard deviatie scores, parameters van botomzetting die onafhankelijk zijn van de nierfunctie en de lichaamssamenstelling veranderden niet bij de patiënten die niet behandeld werden met groeihormoon. Conclusies van dit onderzoek zijn dat de gemiddelde botdichtheid van kinderen met chronische nierinsufficiëntie niet verschilde van gezonde controles. De botdichtheid bleef stabiel, zowel met als zonder groeihormoonbehandeling. Adequate behandeling met alfa-calcidol heeft mogelijk osteopenie bij deze patiënten voorkomen.

In *Hoofdstuk 8* worden de botdichtheid, het botmetabolisme, de lichaamssamenstelling en het vetmetabolisme geëvalueerd bij kinderen met groeihormoondeficiëntie voor en tijdens behandeling met groeihormoon gedurende twee tot drie jaar. Veertig kinderen met groeihormoondeficiëntie, met een gemiddelde leeftijd van 7.9 jaar, participeerden in de studie van de botdichtheid, botmetabolisme en lichaamssamenstelling en een additionele groep van 17 kinderen met groeihormoondeficiëntie, met een gemiddelde leeftijd van 7.9 jaar, in de studie van de vetstofwisseling. De botdichtheid van de lumbale wervelkolom, de volumetrische botdichtheid van de lumbale wervelkolom en

de botdichtheid van het hele skelet waren allen vóór het begin van de behandeling significant verlaagd. Tijdens de groeihormoonbehandeling namen alle botdichtheid variabelen significant toe, de botdichtheid van de lumbale wervelkolom al na zes maanden. De lengte en de massa vetvrij weefsel bleven toenemen tijdens de behandeling. De vetmassa nam gedurende de eerste zes maanden van behandeling af en bleef daarna stabiel. De biochemische parameters van botformatie en botresorptie verschilden niet van normaal voor het begin van de behandeling en stegen gedurende de eerste zes maanden van de therapie. Serum 1,25 dihydroxyvitamine D nam continu toe tijdens groeihormoonbehandeling en de toename had een positieve correlatie met de verandering van de lengte en 'insulin-like growth factor I' standaard deviatie scores. Na drie jaar behandeling bleek de atherogene index te zijn afgenomen en het apolipoproteïne A1 toegenomen, waaruit blijkt dat groeihormoonbehandeling een positief effect heeft op het lipidenprofiel.

In *Hoofdstuk 9* worden de belangrijkste determinanten van de botdichtheid op de kinderleeftijd nog eens toegelicht. De resultaten van het onderzoek naar lichaamssamenstelling en botmetabolisme worden geëvalueerd en de klinische implicaties worden besproken. Aanbevelingen en suggesties voor toekomstig onderzoek naar preventie en behandeling van een lage botdichtheid bij kinderen worden gegeven.

## Abbreviations

| | |
|---|---|
| ALL | acute lymphoblastic leukemia |
| Apo-A1 | apolipoprotein A1 |
| Apo-B | apolipoprotein B |
| BIA | bioelectrical impedance analysis |
| BMD | bone mineral density |
| BMAD | bone mineral apparent density |
| BMI | body mass index |
| CA/CR | ratio of calcium and creatinine in urine |
| CPP | central precocious and early puberty |
| CRF | chronic renal failure |
| DXA | dual energy x-ray absorptiometry |
| FFA | free fatty acids |
| GH | growth hormone |
| GHD | growth hormone deficiency |
| GHRx | growth hormone treatment |
| GnRH | gonadotrophin-releasing hormone agonist |
| HDL | high-density lipoprotein cholesterol |
| ICTP | cross-linked telopeptide of type I collagen |
| IGF-I | insulin-like growth factor 1 |
| IGFBP-3 | insulin-like growth factor binding protein 3 |
| ICS | inhaled corticosteroids |
| LDL | low-density lipoprotein |
| OHP/CR | ratio of hydroxyproline and creatinine in urine |
| PICP | carboxyterminal propeptide of type I collagen |
| PTH | parathyroid hormone |
| QMD | quantitative microdensitometry |
| SDS | standard deviation score |
| $SDS_{BA}$ | standard deviation score calculated for bone age |
| TC | total cholesterol |
| TG | triglycerides |
| VLDL | very low-density lipoprotein |

## Dankwoord

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## About the author

Annemieke M. Boot was born in Arnhem on August 26, 1962. She passed grammar school in 1980 and started medical school at the University in Utrecht. In 1982 she spent one month with a general practitioner in Inverness, Scotland, and in 1984 four months in hospitals of Nairobi, Mumias and Alupe in Kenya and of Masaka in Uganda for clinical experience. In December 1987 she obtained her medical degree. She worked at a casualty department in the centre of Amsterdam for a few months. From September to December 1988 she attended a course in Tropical Medicine and Hygiene at the Tropical Institute in Amsterdam. She thereafter got experience in surgery, obstetrics, paediatrics and internal medicine at Queen Elizabeth Central Hospital, Blantyre, Malawi from January 1989 to May 1990. The following two years she worked as a clinician and district health officer at the District Hospital of Mangochi, Malawi. In September 1992 she started research in bone mineral density and body composition in children at the division of Endocrinology, Sophia Children's Hospital, in collaboration with the department of Nuclear Medicine and department of Internal Medicine III of the Dijkzigt Hospital. Since October 1997, she has started specialist training in paediatrics at Sophia's Children's Hospital in Rotterdam (head: Prof. Dr. H.A. Büller). She is married to Alewijn Ott, epidemiologist and microbiologist-in-training. They have a daughter called Susan.

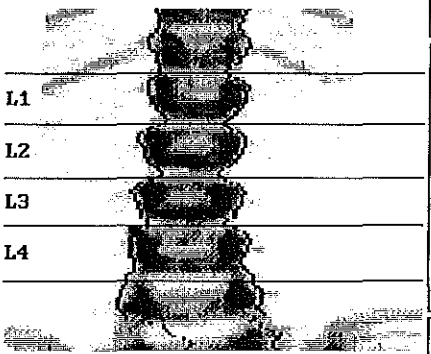
# Appendix

A result of bone mineral density measurement of the lumbar spine and total body, and assessment of body composition, with dual energy x-ray absorptiometry.

**DPX WERVELKOLOM**
**AZR-DIJKZIGT**
afd. Nucleaire Geneeskunde Tel 0104633737

PATIENT ID:
NAME:

SCAN: 1.3z 03/11/96
ANALYSIS: 1.3z 03/11/96




LUNAR®

IMAGE NOT FOR DIAGNOSIS

ID:

SCAN DATE: 03/11/96

Reference Data Not Available

L2-L4 BMD ($g/cm^2$)[1] 0.752 ± 0.01

| | | | | | |
|---|---|---|---|---|---|
| Age (years)..... | 10 | Large Standard..... | 274.49 | Scan Mode..... | Medium |
| Sex..... | Male | Medium Standard..... | 204.58 | Scan Type..... | DPX-L |
| Weight (Kg)..... | 31.0 | Small Standard..... | 145.02 | Collimation (mm)..... | 1.68 |
| Height (cm)..... | 134 | Low keV Air (cps)... | 688101 | Sample Size (mm)..... | 1.2x 1.2 |
| Ethnic..... | White | High keV Air (cps).. | 460926 | Current (uA)..... | 750 |
| System..... | 6354 | Rvalue (%Fat)..... | 1.389( 4.2) | | |

| REGION | BMD[1] $g/cm^2$ | Young Adult[2] % | Young Adult[2] Z | Age Matched[3] % | Age Matched[3] Z |
|---|---|---|---|---|---|
| L1 | 0.684 | - | - | - | - |
| L2 | 0.737 | - | - | - | - |
| L3 | 0.810 | - | - | - | - |
| L4 | 0.720 | - | - | - | - |
| L1-L2 | 0.711 | - | - | - | - |
| L1-L3 | 0.745 | - | - | - | - |
| L1-L4 | 0.737 | - | - | - | - |
| L2-L3 | 0.773 | - | - | - | - |
| L2-L4 | 0.752 | - | - | - | - |
| L3-L4 | 0.758 | - | - | - | - |

1 - See appendix E on precision and accuracy. Statistically 68% of repeat scans will fall within 1 SD.
2 - USA AP Spine Reference Population, Ages 20-40. See Appendices.
3 - Matched for Age, Weight(males 50-100kg; females 35-80kg), Ethnic.

**DPX WERVELKOLOM**
**AZR-DIJKZIGT**
afd. Nucleaire Geneeskunde Tel 0104633737

PATIENT ID:
NAME:

SCAN: 1.3z 03/11/96
ANALYSIS: 1.3z 03/11/96

ANCILLARY SPINE RESULTS**

| Region of Interest | BMC (grams) | Area ($cm^2$) | Width (cm) | Height (cm) | BMC/W (g/cm) | Volumetric Density[1] |
|---|---|---|---|---|---|---|
| L1 | 4.79 | 7.00 | 2.92 | 2.40 | 1.64 | 34 |
| L2 | 5.61 | 7.62 | 3.02 | 2.52 | 1.86 | 45 |
| L3 | 6.12 | 7.56 | 3.32 | 2.28 | 1.85 | 60 |
| L4 | 7.39 | 10.25 | 3.88 | 2.64 | 1.90 | 41 |
| L1-L2 | 10.40 | 14.62 | 2.97 | 4.92 | 3.50 | 39 |
| L1-L3 | 16.52 | 22.18 | 3.08 | 7.20 | 5.36 | 46 |
| L1-L4 | 23.91 | 32.43 | 3.30 | 9.84 | 7.25 | 45 |
| L2-L3 | 11.73 | 15.18 | 3.16 | 4.80 | 3.71 | 52 |
| L2-L4 | 19.12 | 25.43 | 3.42 | 7.44 | 5.59 | 48 |
| L3-L4 | 13.51 | 17.81 | 3.62 | 4.92 | 3.73 | 49 |

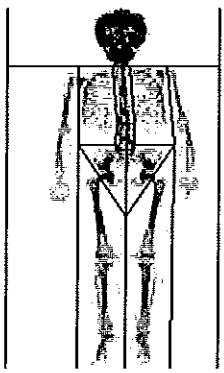
Z-SCORE FOR VERTEBRAL HEIGHT (L2-L4)
Compared to young adult: Z = -7.32
Adjusted for stature: Z = -3.75

**Ancillary results for research purposes, not clinical use.
1 Mazess, et al., 1991, Calc. Tiss. Intl., 48:380-386.

TOTAL BODY
AZR-DIJKZIGT
afd. Nucleaire Geneeskunde tel 0104633737

PATIENT ID:
NAME:

SCAN: 1.3z 03/11/96
ANALYSIS: 1.3z 03/11/96



LUNAR®

IMAGE NOT FOR DIAGNOSIS

ID: 3.988.695 SCAN DATE: 03/11/96

Reference Data Not Available

TOTAL BMD (g/cm²)[1] 0.836 ± 0.01

| | | | | | |
|---|---|---|---|---|---|
| Age (years)..... | 10 | Large Standard..... | 274.49 | Scan Mode..... | Fast |
| Sex..... | Male | Medium Standard..... | 204.58 | Scan Type..... | DPX-L |
| Weight (Kg)..... | 31.0 | Small Standard..... | 145.02 | Collimation (mm)..... | 1.68 |
| Height (cm)..... | 134 | Low keV Air (cps)... | 688101 | Sample Size (mm)..... | 4.8x 9.6 |
| Ethnic..... | White | High keV Air (cps).. | 460926 | | |
| System..... | 6354 | Rvalue (%Fat)..... | 1.369(11.8) | | |
| Current (uA)..... | 150 | | | | |

| REGION | BMD[1] g/cm² | Young Adult[2] % | Young Adult[2] Z | Age Matched[3] % | Age Matched[3] Z |
|---|---|---|---|---|---|
| HEAD | 1.536 | - | - | - | - |
| ARMS | 0.558 | - | - | - | - |
| LEGS | 0.752 | - | - | - | - |
| TRUNK | 0.689 | - | - | - | - |
| RIBS | 0.593 | - | - | - | - |
| PELVIS | 0.765 | - | - | - | - |
| SPINE | 0.764 | - | - | - | - |
| TOTAL | 0.836 | - | - | - | - |

1 - See appendix E on precision and accuracy. Statistically 68% of repeat scans will fall within 1 SD.
2 - USA Total Body Reference Population, Ages 20-45. See Appendices.
3 - Matched for Age, Weight(males 50-100kg; females 35-80kg), Ethnic.
- Standard Analysis.

**TOTAL BODY**
**AZR-DIJKZIGT**
afd. Nucleaire Geneeskunde tel 0104633737

| | | | |
|---|---|---|---|
| PATIENT ID: | | SCAN: | 1.3z 03/11/96 |
| NAME: | | ANALYSIS: | 1.3z 03/11/96 |

BODY COMPOSITION**

| Region of Interest | R Value | Tissue % Fat | Region % Fat | Tissue (g) | Fat (g) | Lean (g) | BMC (g) |
|---|---|---|---|---|---|---|---|
| LEFT ARM | 1.365 | 13.6 | 13.1 | 1406 | 191 | 1215 | 55 |
| LEFT LEG | 1.360 | 16.1 | 15.6 | 5111 | 823 | 4288 | 158 |
| LEFT TRUNK | 1.376 | 8.1 | 7.9 | 6876 | 558 | 6318 | 170 |
| LEFT TOTAL | 1.369 | 11.7 | 11.3 | 15117 | 1769 | 13347 | 556 |
| RIGHT ARM | 1.379 | 7.0 | 6.7 | 1367 | 95 | 1272 | 53 |
| RIGHT LEG | 1.359 | 16.8 | 16.3 | 4966 | 833 | 4134 | 157 |
| RIGHT TRUNK | 1.375 | 8.7 | 8.5 | 6671 | 580 | 6092 | 173 |
| RIGHT TOTAL | 1.368 | 12.0 | 11.6 | 14783 | 1774 | 13009 | 564 |
| ARMS | 1.372 | 10.0 | 9.7 | 2773 | 278 | 2495 | 108 |
| LEGS | 1.359 | 16.4 | 15.9 | 10077 | 1656 | 8421 | 315 |
| TRUNK | 1.375 | 8.4 | 8.2 | 13547 | 1137 | 12410 | 342 |
| TOTAL | 1.369 | 11.8 | 11.4 | 29900 | 3543 | 26357 | 1120 |

ANCILLARY TOTAL BODY RESULTS**

| | |
|---|---|
| Total Bone Calcium (g).. | 426 |
| Air Points..... | 12052 |
| Tissue Points..... | 6756 |
| Bone Points..... | 2909 |
| Total Points..... | 18840 |
| R-Value Points..... | 2542 |

Cut Locations

| Name | Actual | Relative |
|---|---|---|
| Neck | 27 | 27 |
| Left Arm | - | - |
| Left Rib | - | - |
| Right Rib | - | - |
| Right Arm | 80 | - |
| Spine | 50 | 50 |
| Pelvis | 59 | 59 |
| Top of Head | 0 | |
| Center | - | |

**Ancillary results for research purposes, not clinical use.
Standard Analysis.