INVESTIGATION OF BONE METABOLISM IN HEALTHY 6-18 YEARS OLD CHILDREN

PhD thesis

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Introduction

Understanding the complex process of growth and development and control of optimal development as well as the earliest recognition the abnormal development is essential in pediatrics. It is likewise important regarding the changes of the bone metabolism in children. During the period of bone modelling the bone reaches the adult size and mineral content. Peak bone mass is acquired by 25-30 years of age, genetic factors may account for 70% of bone mass and environmental factors may influence some 30%. In childhood bone development is a complex process. Optimal development requires age-adequate quantities of protein, calcium, micronutrients, vitamin D-intake, regular exercise, cytokines, growth factors, and optimal levels of parathyroid, insulin-like growth factor-1, thyroid, leptin, gonadal and pituitary hormones. Gonadal steroids and leptin promote chondrocyte maturation and estrogens act on osteoblasts by stimulating their proliferation and differentiation, increasing osteoprotegerin (OPG) production and decreasing RANK ligand (receptor activator of nuclear factor-κB) production. Growth hormone (GH) increases bone formation through inducing the production of IGFI and somatomedins. Thyroid hormones also stimulate IGFI production. In adults the continuous bone re-modelling (bone resorption and formation) occurs. Peak bone mass is nearly constant untill 50 years of age, after this the bone resorption is increasing with remarkable differences between sexes (3-4%/year in women, 0,3%/year in men).

Assessment of bone development in childhood is often challenging, because the rate of bone accrual depends on the gender, age and Tanner stage of pubertal development. Pubertal development is assessed by Tanner criteria in both sexes. In girls the pubertal growth begins 2 years earlier than boys, their peak bone mass increases by approximately 50 %, in boys nearly 15%. The rate of bone accrual starts to decrease in girls at 15 years of age, in boys at 16 years of age, and it comes to a complete stop in girls at the age of 18, while in boys nearly at the age of 20. The gender differences in bone accrual and body composition become more obvious.

Dual energy X-ray absorptiometry (DXA) technique is widely used for measurements of bone mass, DXA calculates areal bone mineral density (aBMD, gr/cm^2). Z-score is calculated from the actual BMD values the which compares the actual BMD to gender and age-matched reference data, normal reference values are: ±2,0 SD. In childhood whole body DXA technique is suggested, it is independent from body sizes bone density (axial and peripheral bones) total and regional body fat, as well as lean tissue mass and its effective radiation dose is only 0,04 μS. The different climate, lifestyle and genetic factors should be taken considered when evaluating BMD parameters and the use of national reference values is recommended.

BMD values reflect the static parameters of bone tissue, however biochemical parameters provide a dynamic picture of bone metabolism. In practice, as markers of bone formation, osteocalcin (OC) synthesised by osteoblasts and procollagen type 1 N terminal propeptide (P1NP) also formed by osteoblasts reflects rate of collagen and bone formation, while as markers of bone resorption, beta-crosslaps (β-Cl) are widely used. National childhood reference range based on gender and age play a key role in the adequate assessment.

In childhood forms of primer osteoporosis or more accurately, low bone mineral content are very rare: osteogenesis imperfecta, idiopathic juvenile osteoporosis, osteoporosis pseudoglioma syndrome. Secondary osteoporosis can affect at-risk groups of children. It these cases the optimal bone development and the optimal peak bone mass is compromised by environmental factors (inadequate nutrition, life style), by the consequences of the numerous chronic disorders or their treatments. The imbalance of bone formation and resorption is caused mainly by endocrinological, gastrointestinal, chronic liver and kidney diseases, endocrinological, gastroenterological, haemato-oncological, autoimmune disorders and chronic liver and kidney disorders. Based on national data the prevalence of chronic disorders is increasing (in case of coeliac disease 1:85/ child population) and the onset is at younger age wordwide [incidence rate of type 1 diabetes mellitus in children under 5 years is rising by (5.2 %/year)]. In secondary forms bone density assessment and biochemical measurements at the time of diagnosis for the adequate follow-up are recommended.

Prevention has high priority in pediatrics, since the earlier the health protection of youth starts the more effective it is. Our duty is to reach and save the more optimal peak bone mass of the future generations either healthy or suffering from chronic disorders by using the recent densitometric and biochemical measurements of bone metabolism.

Aims

In the western part of Transdanubia the densitometric measurements are performed by German reference values and to assess biochemical parameters childhood reference ranges are not available. For early diagnosis and therapy of bone metabolic diseases national childhood reference ranges based on age and gender are required. Our aim was to investigate bone metabolism parameters in healthy children and to follow the dynamic change of puberty according to Tanner stages in three schools of Szombathely.

1 **Determination of reference range of lumbal bone mineral content and bone biomarkers and assessment of dietary habits and physical exercise in 6-18 years old children**

2 **Determination of reference range of total body mineral content, body composition and serum P1NP values in 6-16 years old children**

3 **Association of body composition, bone biomarkers, gonadal steroids with total and lumbal bone mineral density**

4 **Assessment of vitamin-D status in 6-10 years old children**

PATIENTS AND METHODS

The study protocol was approved by the Regional Medical Ethics Committee. Upon enrolment, informed written consent was obtained from the parents of the children, in accordance with the Declaration of Helsinki. Investigations were performed in the three age-groups at different times: I. age-group (2008. April-May), II. age-group (2005. April-May), III. age-group (2003. October-2004. January) in Children's Endocrinology Polyclinic of Vas County Markusovszky Hospital. Bone mass measurements were performed by DEXA Medical Systems Prodigy (Lunar, Health Care USA). Biochemical parameters were determined by electrochemiluminescence immunoassays using an Elecsys 2010 analyzer from Roche Diagnostics GmbH. Statistical data were analyzed using System of ROPstat except for paragraph 3. Descriptive statistics were presented as the mean and SD or median and quartiles for normally and non-normally distributed characteristics. Comparisons between genders were performed by an independent -sample t test or Mann-Whitney test. p values less than 0.05 were considered significant.

1. Four-hundred-six children (175 boys, 231 girls) healthy, not suffering from chronic diseases mean age: 13,5±2,8 years were enrolled in the study. The parents filled out a questionnaire on background characteristics, medical history and lifestyle factors (nutrition, soft drink consumption, physical activity). The daily calcium and vitamin D intakes were estimated of nutritive contents of all foods and supplements. According to three age-groups the recommended dietary intake of calcium was 800 mg/day for children 6-10 years, 1,000 mg/day for children 11-14 years and 1200 mg/day for children 14-18 years. Consumption of soft drinks and coke, physical activity (regular, and occasional and/or at school) were also estimated. The study population did not participate in any special sports education programme, also nobody was excepted from regular school sport activities. Anthropometric measurements (height, weight), body mass index (BMI) and BMI Z-scores were calculated according to the National Growth Chart and Guide. Pubertal development was assessed by using Tanner criteria. Lumbal (L1-L4) BMD and bone mineral content [BMC, (gr)] were measured. Serum OC (bone formation) and β-Cl (bone resorption) were determined.

2. Two hundred and thirty-seven children (104 boys, 133 girls) in the age-group (I-II.), mean age: 11.6±2.2 years were enrolled in the study. Total BMD, BMC and parameters of body composition were also measured by DEXA, including fat body mass (kg), total lean body mass (kg), and appendicular lean body mass [bone-free lean mass in the arms and legs (kg)]. Indexes of body composition were calculated, including total fat mass index (FMI kg/m2) and total body and appendicular lean mass index (LMI kg/m2). In addition to the previous biochemical investigations the age and gender specific reference range of serum P1NP, another marker of bone formation was determined.

3. Study population (n=237) and the osteodensitometric measurements were the same as in point two. Serum OC, β-Cl, P1NP, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) intact parathyroid hormone (PTHi), estradiol (E2), total testosterone (T), were determined. The association of bone mass with body composition, bone turnover markers, gonadal steroids, PTHi were examined. Statistical data were analyzed using IBM SPSS software (version 20.0). Associations between variables were evaluated using univariate and stepwise multiple linear regression. In the study the dependent variables were BMD (total and L1-L4) and independent variables were OC, gonadal steroids (E2, T) and parameters of body composition (fat mass index and appendicular LMI).

4. We studied serum 25-hydroxyvitamin D3 [25(OH)D3], PTHi and vitamin-D intake by questionnaire of eighty-six (43 boys) prepubertal (Tanner stage I) children. The vitamin D status was determined by assessing 25-hydroxy-D_3 concentrations (25(OH)D_3) using cut off values according to the last recommendation. We defined 25 (OH)D deficiency as <50 nmol/l, insufficiency as 50-75 nmol/l, and optimal as >75 nmol/l in sera. According to Hungarian guidelines which recommends to stop vitamin D supplementation at three age, we studied the effect of vitamin-D supplementation on vitamin D status and bone mass.

RESULTS AND CONCLUSION

1. Determination of reference range of lumbal bone mineral content and bone biomarkers and assessment of dietary habits and physicial exercise in 6-18 years old children [1,3,5]*

* Numbers in parenthesis refer to related own publications.

1 In the age-group I, prepubertal boys (6-10 years) were significantly taller than girls, however in girls lumbal BMD and serum OC levels were significantly higher reflecting the earlier onset of puberty in girls.

In the age-group II.(11-16 years) anthropometric parameters were not significantly different between genders. Significantly higher L1-L4 BMD, BMC values in girls, and serum OC, β-Cl values in boys correspond to the later start but prolonged duration and higher bone mass accumulation in boys.

In the age-group III. (14-18 years) all investigated parameters (anthropometric, osteodensitometric (L1-L4 BMD, BMC), serum OC, β-Cl of boys were significantly higher than girls. Increased lumbal bone mass accumulation in both genders reflected the pubertal growth spurt.

2 **Assessment of lumbal BMD, BMC parameters according to gender, age and Tanner stages**

L1-L4 spine densities were accelerating at age 11 and higher significant accumulation was seen at age 12-14 in girls. In boys lumbal densities were increased at age 13, then values exceeded the girls' densities at age 17, and further accumulation was observed at age 18.

3 **Assessment of lifestyle habits in children and adolescents**

Calcium and Vitamin-D intake

Calcium is a macroelement, it is essential for optimal bone hardness, strength and also called as the substrate of vitamin D. Mean calcium intake was above the Hungarian recommendations in all three age-groups. However the mean vitamin D intake was largely below the requirements by age (400 NE vs. 1000 NE/day).

Soft drink consumption

In school children the coke consumption is less widespread; other carbonated soft drinks are more popular. At the age-group II, more than third of adolescents were coke- and soft drinks consumers. In the age-group III. more than half of boys were regular coke-consumers, compared to one third of girls. About half of adolescents were other soft drinks-consumers in both gender.

Assessment of physical activity in different age-groups

More than half of school children were involved in competitive sporting activities, however in the age-group II the sporting intention was decreasing in both genders, about third of boys and only 40 % of girls did sports regularly. Further decreasing was estimated in the age-group III. Only third of

boys were involved in regular sporting activity, however only 20 % of girls were played competitive sport.

2. Determination of reference range of total body mineral content, body composition and serum P1NP values in 6-16 years old children

1. **Assessment of TBMD, TBMC values according to age, gender and Tanner stages**

Slight increase of TBMD values were detected in both gender up 10 years old. TBMD parameters of 12 years old girls were significantly higher than of boys. Only at age of 16 years the boys TBMD values were higher. Considerable increasing of TBMC values were observed at both genders from 8 years old, later the parameters of 11-year-old girls were higher, the parameters of boys only at age of 14 exceeded the girls' measurements.

2. **Assessment of body composition parameters according to age, gender and Tanner stages**

Continuously increased fat mass values were observed in girls, peak values were at 16 years old. In boys slight increase was shown at age of 15. Values of lean body mass (total and appendicular) rose up 13 years old at both genders, in boys in direct correlation with Tanner stage and peak values were at 16 years. Our data of body composition are in accordance with gender differences. Childhood reference range of body composition measuring by DXA based on age, genders and Tanner stages is not available in our region.

3. **Determination of serum bone biomarkers (OC, β-Cl, P1NP) according to age, gender and Tanner stages**

Serum values of OC, β-Cl, (I-III. age-groups), P1NP (I-II. age-groups) at the same age, but at different Tanner stages were different reflecting the changes in rapid bone development. In girls a peek of all three bone turnover markers were detected at age of 11-12 years, these values in boys correspond to the two years later start but longer duration and higher bone mass accumulation. At age 13 in girls, when growth velocity declines, bone marker levels also decrease. In boys it is observed only at age of 14-15 years, and the degree of decline is less. Close to adulthood growth velocity declines, bone marker levels decrease but bone mass still increases.

3. Association of bone mass parameters, gonadal steroids, body composition with total and lumbal BMD [8]

The regulation of bone metabolism from childhood to puberty is a complex process, influenced by numerous factors, therefore we scrutinized the physiological differences in bone development at different pubertal stages. During the prepubertal period, gender differences in body composition were not visible by physical examination alone; the osteodensitometric parameters were different

between genders. In puberty OC had a significant negative effect on L1-L4 BMD in boys and on BMD (total and lumbar) parameters in girls. The decline of the levels of bone turnover markers begins and stops earlier in girls, and the negative effect on bone mass reflects the decline of pubertal growth spurt. In boys significant negative effect of OC on lumbal BMD corresponds to later start but prolonged duration of puberty in boys. In prepubertal girls significantly higher PTHi levels within the normal reference range may coincide with the earlier initiation of pubertal growth. In our examination, a significant positive relationship was confirmed between fat mass index and BMDs in girls in both Tanner stage groups. Estradiol levels significantly influenced bone mass in girls in both Tanner stage groups and in midpubertal boys. However, the most significant predictor of bone mass was appendicular LMI in both genders during both pubertal stages.

4. Assessment of vitamin D status in 6-10 years old children

Despite the significantly higher serum 25(OH)D3 concentrations in boys, the recommended optimal level of vitamin D3 (>75 nmol/l) was detected only in16 % of boys. The vitamin D-intake was only 200 NE/day in more than 75% of prepubertal children, compared to the recommended dietary intake of vitamin D (400 NE/day). Low vitamin D-intake and suboptimal serum 25(OH)D3 levels draw attention to low dairy products consumption, which nowadays is very common. Based on our results we recommend to supplement foods with vitamin D and determine the optimal amount and duration of vitamin-D supplementation.

CONCLUSIONS

In our study we determined the reference ranges for densitometry, body composition, bone biomarkers according to gender, age and Tanner stages in healthy 6-18 years old children and adolescents. Bone mass parameters (biochemical, densitometric, body composition measurements) correlated with gender differences during the onset of the puberty of the pubertal growth spurt. We have shown that if bone mass parameters of children at the same age, but at different Tanner stages are evaluated the Tanner stage matched reference range should be used. The lower bone densitometric or biochemical parameters should be differently evaluated in prepubertal children and in children already at the onset of puberty. In the pubertal children increase of bone mass parameters expected. The complex effect of numerous factors on bone mass was shown in both genders during both pubertal stages.

The most significant predictor of bone mass was appendicular LMI, but the role of estrogen, testosterone and fat mass index in girls were also important. In puberty we proved that the OC had a significantly negative effect on L1-L4 BMD in boys and on BMDs in girls, which is explained by the almost complete pubertal development is girls and the gradually decelerating growth velocity in boys.

Low vitamin D-intake and suboptimal serum 25(OH)D3 levels of prepubertal children draw attention to the need of reevaluation of the Hungarian professional recommendation for the vitamin D supplementation, and -for the classical and so called non-skeletal benefits of Vitamin D- the fortification of foods with Vitamin D can also be suggested in order to achieve more optimal peak bone mass of the future generations. Our data help to determine the Hungarian reference range of osteodensitometric and biochemical parameters and to introduce them in the medical practice.

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