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Hormonal and Bone Parameters in Pubertal Girls

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Summary

Here we analyzed associations between muscles mass, total bone mineral content (BMC), lumbar spine bone density (BMD L1-L4) and serum or urine hormones in healthy peripubertal girls. Total BMC and areal BMD L1-L4, muscle mass and fat were measured by dual-energy X-ray absorptiometry (DXA). Muscle force (N) was estimated by a dynamometer. Circulating estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), 25-hydroxy vitamin D, parathyroid hormone (PTH), insulin-like growth factor 1 (IGF-1), leptin, osteocalcin, bone isoenzyme of alkaline phosphatase (bALP) and total calcium and phosphorus were quantified as the nocturnal melatonin and serotonin urinary excretion. Partial correlations adjusted for height, Tanner score and physical activity confirmed positive relationships between BMC or BMD L1-L4 (Z-score) and lean mass or fat. Furthermore, positive relationship was observed between BMC or BMD L1-L4 (Z-score) and serum leptin. After adjustment for Tanner score and physical activity, positive associations were observed between lean mass and IGF-1, leptin levels or muscle force. We proved positive relationships between bone mass and serum leptin in peripubertal girls.

Key words

Bone • Puberty • IGF-1 • Leptin • Serotonin • Melatonin

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Introduction

It is known that the development and status of bones depends not only on the classical hormonal system regulating calcium phosphate metabolism (parathyroid hormone, calcitonin, vitamin D and its metabolites) but also on hormones with primary different effect as melatonin, serotonin, leptin and other adipokines.

Recently, there is a considerable interest generated about the interaction of melatonin and bone metabolism. Although, melatonin plays a key role in the circadian timing system, has a variety of biological actions such as regulation of seasonal reproductive function, immunity, antitumor and antioxidant effects, the osteogenic properties of the hormone still remain not fully elucidated (Park *et al.* 2011, Man *et al.* 2011, Satomura *et al.* 2007, Sethi *et al.* 2010). Accumulating evidence from *in vitro* and *in vivo* experiments using animal models has also suggested that melatonin may have an influence on skeletal growth, bone remodeling and to play a role in the parathyroid function. It has been reported that individuals with abnormalities in melatonin secretion, especially in the nocturnal phase, demonstrate considerable bone mass loss and also abnormalities of bone metabolism; the pivotal role of melatonin, estrogens and androgens on bone formation has also been underlined in these reports (Leder *et al.* 2003, Riggs *et al.* 2002, Manolagas *et al.* 2002). The early differentiation and higher expression of bone marker proteins and also the osteogenic differentiation on bone marrow stem cells are evoked by the presence of melatonin (Cardinali *et al.* 2003, Zaminy *et al.* 2008). Follicle stimulating hormone

(FSH) and luteinizing hormone (LH) seem to also influence the bone mineral status (Kancheva *et al.* 2012).

The bone is a tissue which not only produces but is also influenced by many hormones. The effect of estrogens, androgens, growth factors, active metabolites of vitamin D and some other hormones on bone remodeling are connected to receptor activator of nuclear factor-κB ligand/activator of nuclear factor-κB/ osteoprotegerin (RANKL/RANK/OPG system) modulation. This signaling pathway regulates the osteoclasts and osteoblasts activity as well as mediates the effects of the hormones on the skeleton (Ostrowska 2009). The concept of muscle – bone unit assumes that bones and muscles represent evolutional functional unit that is under the control of insulin-like growth factor 1 (IGF-1), gonadal hormones and vitamin D (Ashby *et al.* 2011, Fricke *et al.* 2010). The anabolic effect of IGF-1 on bone is well known and this is true during the puberty and adolescence (Mohan and Baylink 2005, Venken *et al.* 2005). The variation of IGF-1 expression is one of the causes of inter-individual differences of peak bone mass values (Karatay *et al.* 2007, Maimoun *et al.* 2010).

Vitamin D is a hormone which is involved in the regulation of bone development. It was reported that hypovitaminosis D, characterized by extremely low levels of 25-hydroxy vitamin D [25-(OH)D] decelerates muscle and bone development significantly more in girls than in boys, especially when associated with low physical activity (Ward *et al.* 2009). However, the interrelationship between vitamin D and muscle – bone unit in peripubertal girls has not been extensively studied.

Inconsistent data are available in the literature regarding the role of adipokines as well as serotonin – melatonin axis in bone development (Cirmanova *et al.* 2008). Leptin activates osteoblasts´ receptors and thus directly increases bone mass accrual. However, leptin also involves central inhibitory effect on the skeleton *via* β_2 receptors that activate bone turnover (Hipmair *et al.* 2010).

Recently, research focused on the osteotropic effects of the neurotransmitter 5-hydroxytryptamin (serotonin). Dampening of serotonin production induced by Wnt/Lrp/beta-catenin circuit activation has an anabolic effect on bones (Yadav *et al.* 2010). In addition, some authors pointed out that higher serotonin tissue levels could explain the increased incidence of fractures in patients treated with antidepressants, inhibitors of the serotonin reuptake (Warden *et al.* 2010).

The exact role of melatonin on pubertal bone development is not elucidated. The indole inhibits production of gonadotropins (Srinivasan *et al.* 2009). On the other hand, it has direct bone anabolic effect which is mediated by specific receptors on the osteoblasts, inhibition of adipocytogenic differentiation and activation of osteogenesis (Radio *et al.* 2006, Sanchez-Hidalgo *et al.* 2007, Suzuki *et al.* 2008, Witt-Enderby *et al.* 2006, Zhang *et al.* 2010). To the best of the authors' knowledge, the role of serotonin – melatonin system in the regulation of bone development has not been extensively investigated so far.

The aim of the study was to investigate the relationships between hormones, soft tissues and total bone mineral content (BMC) and lumbar spine bone mineral density (BMD L_1-L_4) in healthy peripubertal girls.

Methods

Subjects

A total of 100 healthy school girls randomly selected from several Prague regions were examined. The inclusion criteria for participation in the study were: age 9-15 (average 12.3±1.36) and Tanner stage 1-5 (average 2.3). Exclusion criteria were: malnutrition, milk intolerance, any known internal or psychiatric diseases or medicament treatment. Information about physical activity (normal school exercise once a week or recreation sports) and about dietary habits (intake of dairy products), neonatal data (fetal maturity and breast-feeding duration) were obtained by the use of a questionnaire from the parents of the participants. Informed consent was obtained from all the girls as well as their parents and the study was approved by the Ethics Committee of the Institute of Endocrinology in Prague.

Protocol

Blood samples on fasting were obtained to assess estradiol, FSH, LH, parathyroid hormone (PTH), 25(OH)D, IGF-1, leptin, osteocalcin, bone isoenzyme of alkaline phosphatase (bALP), total calcium and phosphorus. Nocturnal melatonin and serotonin urinary excretions were also studied. In all participants, values of total BMC (g), BMD L_1-L_4 (g/cm^2 and its Z-score), lean-muscle and fat mass (g) were evaluated by use of dual-energy X-ray absorptiometry (DXA). Normal BMD in children is considered to be in the range of ±2 SD. The precision of the method is 1 %. Muscle force (N) was estimated by a dynamometer and was expressed as a sum

of isometric flexion and extension of fingers, arms, shanks and flexion and extension of the trunk.

Laboratory parameters

The measurement of estradiol was done by a commercial kit from the HUMA LAB, for FSH and LH HUMA LAB CS kits were also utilized (Krasna, Slovak Republic). 25(OH)D was measured using the RIA kit of IDS Company (The Boldons, UK). Serum PTH was set by the use of electrochemiluminiscence (ECLIA) with Roche analyzer (Cobas 6000, Manheim, Germany). Serum leptin was assessed using RIA kit LINCO Research (St. Charles, MO, USA), serum IGF-1 using IRMA method from IMMUNOTECH (Marseille, France). Night urine melatonin excretion was measured using ELISA immunoassay (IBL Intenational, Hamburg, Germany), similarly as urine serotonin excretion. Total blood calcium and phosphate were measured using absorption spectrophotometry (Cobas 6000, Manheim, Germany). Serum osteocalcin was determined by ECLIA method from Roche (Cobas 6000, Manheim, Germany) and bALP by IRMA kit from Beckman Coulter (Prague, Czech Republic).

Statistics

Mean, standard deviations, median, maximum and minimum were used for description of the investigated variables. To measure the dependence of two variables, while the subgroup of choose variables was held constant, the partial correlation coefficient was used. Normality was tested by Shapiro-Wilk's W statistic. All "p" were two-sided and $p<0.05$ was regarded as significant. Statistical software SYSTAT was used for the calculations.

Table 1. Partial correlations of dependent variables (osteocalcin, bALP, total BMC, BMD L_1-L_4 and BMD Z-score) with independent variables (Ca – calcium, P – phosphate, serum and urine hormones, soft tissues and neonatal parameters) after removing linear effects of height, Tanner score and physical activity.

	Osteocalcin	bALP	BMC total	BMDL1-L4	BMD Z-score
Ca	0.112	-0.061	0.022	0.004	-0.018
P	0.299**	-0,028	-0.032	-0.093	-0.017
25(OH)D	0.252*	-0.089	0.015	-0.001	0.028
1,25(OH)$_2$D$_3$	0.166	-0.062	-0.044	0.132	0.014
PTH	0.227*	0.184	-0.096	-0.111	-0.122
IGF-1	0.174	-0.007	0.090	0.074	0.131
Estradiol	-0.037	-0.303**	0.142	0.143	-0.096
Leptin	-0.214	0.115	0.303*	0.217	0.275*
Melatonin	0.313**	-0.122	-0.138	-0.130	-0.242
Serotonin	0.168	0.234*	-0.232	-0.245	-0.052
Fat	-0.385**	-0.039	0.564***	0.377**	0.482***
Lean	-0.100	-0.004	0.666***	0.188	0.551***
Muscle force	-0.046	0.001	0.349**	0.188	0.242
Gestation	0.125	0.120	0.196	0.194	0.190
Breastfeeding	-0.029	-0.002	0.011	-0.035	-0.061

BMC – total bone mineral content, BMD – bone mineral density, PTH – parathyroid hormone, bALP – bone alkaline phosphatase isoenzyme, 25(OH)D – 25-hydroxy-vitamin D, IGF-1 – insulin-like growth factor 1, *p<0.05, **p<0.01, ***p<0.001.

Results

Table 1 indicates partial correlations between total BMC or BMD L1-L4 (g/cm^2 or Z-score) and independent variables after removing linear effects of height, Tanner and physical activity. Strong positive correlations were found between lean mass and BMC or BMD Z-score ($p<0.001$ and $p<0.001$, respectively), as well as between fat and BMC, BMD L1-L4 and BMD Z-score ($p<0.001$, $p<0.01$ and $p<0.001$). Positive association was observed between leptin and BMC or BMD Z-score ($p<0.05$ for both bone parameters). Positive relationship was noticed between serum phosphate, 25(OH)D, urine melatonin and serum

osteocalcin ($p<0.01$, $p<0.05$ and $p<0.05$, respectively), similarly as between urine serotonin and bALP ($p<0.05$). However, none of these hormonal parameters correlated with BMC or BMD L1-L4.

Table 2. Partial correlations of dependent variables (lean and muscle strength) with independent variables (Ca – calcium, P – phosphate, serum and urine hormones) after removing effect of height, Tanner score and physical activity.

	Lean	Muscle force
Ca	-0.024	-0.013
P	0.163	-0.021
25(OH)D	0.009	-0.212
1,25(OH)$_2$D$_3$	0.049	-0.050
PTH	0.118	0.034
IGF1	0.363**	0.261**
Estradiol	0.043	-0.145
Leptin	0.311*	0.105
Melatonin	-0.046	0.039
Serotonin	-0.067	-0.169

Abbreviations are the same as in Table 1.

Partial correlations between lean or muscle force and hormone indices after removing linear effects of height, Tanner and physical activity are depicted in Table 2, which shows positive associations between the lean or muscle strength and serum IGF-1 ($p<0.01$ and $p<0.01$, respectively) and between lean (but not muscle force) and serum leptin ($p<0.05$). No associations were found between neonatal parameters and BMC or BMD.

Discussion

The study confirmed positive association between pubertal bone and fat and leptin that was also reported by Rhie *et al.* (2010). Thus, both studies support the assumption that the direct stimulating effect of leptin on developing bone mass prevails over leptin inhibitory effect mediated through central mechanism (Hipmair *et al.* 2010).

In addition, a positive relationship was also found between leptin and lean mass values, which is in agreement with the data of Olmedillas *et al.* (2010) who found up-regulation of leptin receptors in hypertrophic biceps of professional tennis players. These results allow raising the hypothesis that the positive effect of leptin on bone is also muscle mediated.

It is acknowledged that muscle function is positively influenced by vitamin D. Ward *et al.* (2009) reported positive correlation between 25(OH)D levels and muscle strength in adolescent girls with a corresponding negative relationship between PTH levels and these parameters. In addition, positive correlations between 25(OH)D and lumbar spine BMD and trochanter BMC were outlined by El-Hajj Fuleihan *et al.* (2006). However, we were unable to prove any association between serum 25(OH)D or PTH and lean or bone mass. These findings could be in some extent explained by the differences in the study participants, i.e. the girls in the study of El-Hajj Fuleihan *et al.* (2006) were undernourished and the participants of the study of Ward *et al.* (2009) were younger than our ones.

It is well known that the female puberty is associated with an increase of gonadal steroid production. Osteotropic effect of estrogens is direct, but also mediated by the IGF-1 (Maimoun *et al.* 2010). In our study relationships between bone parameters and estradiol or IGF-1 levels were shown in unadjusted analysis. However, we were not able to prove these associations after eliminating the effects of age, Tanner score and physical activity. Since IGF-1 production and bone response to the hormone depend also on physical activity (Karatay *et al.* 2007, Maimoun *et al.* 2010) the lacking adjusted-association between serum IGF-1 levels and bone mass could be explained by relatively not pronounced physical activity in our girls. On the other hand, the effect of protein-caloric malnutrition could be eliminated in the vast majority of the girls of our study.

It is established that serotonin influences target tissues (including bones) in a bimodal way depending on the source of its synthesis (duodenum or central nervous system). Serotonin produced by enterochromafin cells inhibits bone formation, while serotonin produced in central nervous system has the opposite effect (Ducy and Karsenty 2010). The lack of association between serotonin levels and bone parameters in our girls could be explained by the different effects of the serotonin pools.

The assumption of the osteo-anabolic effects of melatonin is supported in the present study by proved positive association between nocturnal melatonin excretion and osteocalcin levels. This is in accordance with the recent findings that oral administration of melatonin can increase bone accrual during growth and

can cure ovariectomy-induced structural and functional degeneration of bone by specifically increasing bone formation (Sharan *et al.* 2017). Future studies are needed to look more closely on the bone – melatonin relationship not only in peripubertal girls, but also boys and different age groups of female and male individuals.

Conflict of Interest

There is no conflict of interest.

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