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Relationship Between Hormonal Variables and Bone Mineral Density, Muscle Force, and Fat Mass in Peripubertal Girls

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ABSTRACT

Background: The muscle-bone unit represents an evolutionary system, in which both of its components are under the common control of the insulin-like growth factor I (IGF-I), sex hormones, and vitamin D. The mutual interactions between these hormones maintain integrity, growth and maturation of pubertal bone mass. Thus, insufficiency of any of these hormones will negatively influence development of the skeleton during puberty.
Objectives: The aim of the study as to analyse the correlation between muscle mass, total bone mineral content (BMC), bone mineral density (BMD) of the lumbar spine (BMD L_1-L_4), and serum or urine hormones.
Materials and Methods: Total BMC (g) and areal BMD L_1-L_4 (g/cm^2 and Z-score) as well as muscle mass and fat mass (g) were assessed by means of dual-energy X-ray absorptiometry (DXA). The Z-score is the number of standard deviations a patient´s BMD which differs from the average BMD of their age, sex, and ethnicity. This Parameter is used in children. Muscle force (N) was measured using a dynamometer.
Results: The simple correlations showed strong positive associations between BMC or BMD L_1-L_4 (g/cm^2) and serum phosphate, estradiol, insulin-like growth factor (IGF-I), leptin and fat masses, and muscle force ($P < 0.001$ for all parameters). Positive correlations were also observed between BMD and serum phosphate ($P < 0.01$), IGF-I ($P < 0.01$), estradiol ($P < 0.001$), leptin ($P < 0.01$), fat and lean mass ($P < 0.001$ and $P < 0.001$, respectively) and muscle force ($P < 0.001$). The partial correlations, after eliminating the impact of height, Tanner stage, and physical activity level, confirmed positive relationships between either BMC or BMD L_1-L_4 and lean mass ($P < 0.001$ and $P < 0.001$, respectively) and fat mass ($P < 0.001$ for BMC and BMD). Furthermore, a positive relationship was observed between serum leptin and both BMC and BMD (Z score) ($P < 0.05$ and $P < 0.05$, respectively). After removing the effects of height, Tanner stage, and physical activity, positive associations were observed between lean mass and IGF-I ($P < 0.01$), leptin levels ($P < 0.05$), and muscle force ($P < 0.01$).
Conclusions: On the basis of the study results, it can be expected that low values of lean or fat mass, and insufficient production of IGF-I or leptin, could negatively influence bone development in pubertal girls.

Implication for health policy/practice/research/medical education:
Monitoring of bone mineral density together with muscle indices and hormonal parameters in pubertal girls could prevent development of osteoporosis in women.

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1. Background

A bone is not only a motor apparatus, but also a living tissue, both producing and receiving many hormones. The effects of estrogens, androgens, growth factors, the active metabolite of vitamin D, and many other hormones on bone remodelling are largely related to receptor activator of nuclear factor-κB ligand/receptor activator of nuclear factor -κB/osteoprotegerin (RANKL/RANK/OPG system) modulation. This signalling loop directly regulates the activity of osteoclasts and osteoblasts and mediates the effects of hormones and cytokines on the skeleton (1). The concept of the muscle-bone unit assumes that bones and muscles represent an evolutionary functional unit, and both of its components are under the control of the insulin-like growth factor I (IGF-I), sex hormones and vitamin D (2,3). IGF-I has a direct anabolic effect on bone (4). In mutual interactions with oestrogens and androgens (5) this peptide initiates growth spurts and accelerates maturation of bone mass during puberty and adolescence (6). The highest levels of IGF-I are reported between the 3rd and 4th Tanner stage and corresponding to bone maturity (7). It is expected that variations in IGF-I expression are one of the causes for inter individual differences in peak bone mass values, its quality and dimension of the skeleton in humans with a positive energy balance and adequate physical activity (8).

The next hormone regulating bone development is vitamin D. It was observed that more serious cases of hypovitaminosis D slowed muscle and bone development with the effect being significantly more prevalent in girls than in boys, particularly when associated with low levels of physical activity (9). However, the relationship between D vitamin homeostasis and the muscle-bone unit in peripubertal girls has not been extensively studied, despite a generally high prevalence of hypovitaminosis D in children and adolescents. Inconsistent data have been obtained on the role of adipokines and the serotonin – melatonin axis in bone development. Leptin activates the receptors of osteoblasts and thus, directly increases bone mass accrual. However, leptin also exerts a central inhibitory effect on the skeleton via β_2 receptors, which activate bone turnover (10).

In recent years, attention has been focused on osteotropic effects of neurotransmitter 5-hydroxytryptamine (serotonin). Dampening of serotonin production, induced by Wnt/Lrp/β-catenin circuit activation, has an anabolic effect on bones (11). Furthermore, higher serotonin levels could explain an increased incidence of fractures in patients treated with antidepressants that inhibit serotonin reuptake (12). The relevance of melatonin in pubertal bone development is not clear. It is known, that this indole inhibits production of gonadotropins (13). Conversely, it has direct anabolic effects on bones, mediated by specific receptors on osteoblasts that induce inhibition of adipocytogenic differentiation and activation of osteogenesis (14-18). The authors are unaware of any specific investigations regarding the role of the serotonin - melatonin system in the regulation of bone development.

2. Objectives

The goal of this study was to assess the relationships between hormones, soft tissues, and the total bone mineral content (BMC) (g) or bone mass density (BMD) of the L_1-L_4 vertebras (g/cm^2 or Z-score) in a group of healthy peripubertal girls.

3. Materials and Methods

3.1. Subjects

A total of 100 healthy girls, aged 9-15 years (average 12.3 ± 1.4), randomly selected from several regions in Prague, were examined. Inclusion criteria compromised the following: having reached puberty stage 1-4, having a good nutritional state, and consenting to follow the terms of the study protocol. The stage of puberty was determined according to an assessment of the extent of axial hair (Tanner A), pubic hair (Tanner P) and breast development (Tanner M). Thirty girls were assessed at Tanner 1, thirty-six girls at Tanner 2, ten girls at Tanner 3, and twenty-four girls were in Tanner 4 stage. Forty-two of our girls were menarcheal. Exclusion criteria were as follows: malnutrition, milk intolerance, any internal or psychiatric diseases, or current treatment with medications. Information about physical activity (evaluated as: grade 1, for normal school exercise once a week, grade 2, for regular participation in recreational sports, and grade 3, for those involved in racing sports), dietary habits (intake of dairy products), and neonatal data (fetal maturity at birth and duration of breast feeding) were gained from their parents through the use of a questionnaire. Informed consent was obtained from all of these girls and their parents and all procedures were approved by the Ethics Committee of the Institute of Endocrinology in Prague.

3.2. Protocol

Blood samples were taken from fasting girls in the morning (between 7 and 8 a.m.) to assess estradiol, follitropin (FSH), lutropin (LH), vitamin 25(OH)D, parathormone (PTH), IGF-I, leptin, adiponectin, osteocalcin, bone isoenzyme of alkaline phosphatase (bALP), and total calcium and phosphorus. In all girls nocturnal melatonin

and serotonin excretion was determined in urine collected from 9 p.m. to 6 a.m. To minimize the effect of estradiol fluctuations during the menstrual cycle, samples from the 42 girls, who had attained menarche was performed immediately after menstruation.

In all girls, values of total BMC (g/cm^2), BMD L_1-L_4 (g/cm^2 and its Z-score (referenced to a healthy population of the same age), lean (muscle) and fat mass were measured by use of dual-energy X-ray absorptiometry (DXA). The precision of the method is 1%. Muscle force was measured with a dynamometer (N) and was expressed as the sum of isometric flexion and extension of fingers, arms, lower legs, and the flexion and extension of the trunk.

3.3. Laboratory Studies

Estradiol was estimated using a commercial kit from the HUMA LAB CS. The inter-assay coefficient of variation (CV) was 15%. FSH and LH were measured using HUMA LAB CS kits (Slovak Republic). 25(OH) vitamin D was measured using an RIA kit (Immunodiagnostic System Holdings PLC) (normal range 9.2 - 45.2 ng/mL, CV 9.9%). Serum PTH was determined using electrochemiluminiscence (ECLIA) assay (upper normal limit 65 ng/mL, CV 6.5%). Serum leptin was assessed using an RIA kit (LINCO Research, (St. Charles, MO, USA) (normal range in women 7.4–3.7 ng/mL, CV 4.0%), adiponectin using an RIA kit from LINCO Research (CV 6.9%), serum IGF-I and IGFBP-3 using IRMA methods from IMMUNOTECH (Marseille, France) (normal values of IGF-I in healthy children 114–400 ng/mL depending on stage of puberty, CV 6.8%). Nocturnal urinary melatonin sulphate excretion was measured using an ELISA immunoassay, IBL (Hamburg, Germany) (mean value in healthy adolescents 2.8 μg/h), and a similar method was used for the determination of nocturnal urinary serotonin excretion (normal values

Table 1. Anthropometric and Biochemical Characteristics of the Study Group

	MV	SD	MIN	MED	MAX
Age, y	12.299	1.362	9.267	12.313	15.358
Weight, kg	44.999	11.209	22	44.5	67
Height, cm	153.96	11.226	126.5	150.15	173.8
BMI, kg/m^2	18.791	3.521	12.252	19.56	27.76
Tanner M	2.282	1.151	1	2.5	4
Tanner P	2.212	1.156	1	2.5	4
Tanner A	2.224	1.127	1	2.5	4
Physical activity	2.012	0.681	1	2	3
Muscle force, N	2430	47.8	1253	2502	3750
Gestation, Wk	39.635	1.022	33	37.5	42
Breast feeding, mo	6.282	4.734	0	12	24
Fat, g	13114	6982.5	2064	15404.5	28805
Lean, g	31236	5907.3	20449	36723	42997
BMC total, g	1749.1	456.37	953.6	1810.8	2688
BMD L1-L4, g/cm^2	0.933	0.176	0.643	1.006	1.369
BMD Z-score	0.171	1.025	-2,5	-0.15	2.2
Serum					
Osteocalcin, ug/L	143.4	48.643	48	148.9	249.8
bALP, ug/L	81.376	32.448	15.03	84.365	153.7
Ca, mmol/L	2.475	0.143	2.18	2.515	2.85
P, moll/L	1.463	0.161	1.14	1.5	1.86
25(OH)D, μg/L	18.985	6.901	10.09	28.945	47.8
PTH, ng/L	42.744	12.677	18.5	47.3	76.1
IGF-I, ng/mL	664.72	236.25	240.5	711.3	1182.1
Estradiol, nmol/L	0.204	0.191	0.007	0.487	0.967
adiponectin, ng/mL	13.554	4.983	5.71	18.58	31.45
Leptin, ng/mL	4.952	2.691	0.85	6.04	11.23
Urine					
Melatonin, μg/hour	107.31	106.89	11.4	294.25	577.1
Serotonin, μg/night	131.43	179.14	0.21	470.06	939.9

Table 2. Partial Correlations Between Bone and Body Composition, Muscle force, Physical Activity, and Hormones.

	Osteocalcin	bALP	BMC Total	BMD L_1-L_4	BMD Z- Score
Calcium	Calcium	0.008	-0.099	-0.149	-0.080
Phosphate	Phosphate	0.353 [c]	-0.379 [c]	-0.414 [c]	-0.273 [b]
25(OH)D	25(OH)D	-0.063	-0.120	-0.016	0.125
1,25(OH)2D3	1,25(OH)2D3	0.124	-0.056	0.041	0.040
PTH	PTH	0.133	0.029	0.025	-0.159
IGF-I	IGF-I	0.185 [a]	0.450 [c]	0.489 [c]	0.304 [b]
IGF-IBP3	IGF-IBP3	-0.153	0.463 [c]	0.463 [c]	0.228
Estradiol	Estradiol	-0.449 [c]	0.487 [c]	0.543 [c]	0.319 [c]
Adiponectin	Adiponectin	0.083	-0.176	-0.107	-0.253
Leptin	Leptin	-0.155	0.350 [c]	0.331 [c]	0.288 [b]
Melatonin	Melatonin	0.017	-0.114	-0.064	-0.166
Serotonin	Serotonin	-0.024	-0.001	0.040	-0.019
Fat mass	Fat mass	-0.332 [c]	0.581 [c]	0.499 [c]	0.527 [c]
Lean mass	Lean mass	-0.380 [c]	0.898 [c]	0.809 [c]	0.554 [c]
Muscle force	Muscle force	-0.260 [b]	0.693 [c]	0.643 [c]	0.426 [c]
Physical activity	Physical activity	-0.020	0.165	0.275 [b]	0.259 [a]
Breast feeding	Breast feeding	-0.002	-0.002	-0.020	0.118
Neonatal maturity	Neonatal maturity	-0.020	0.003	0.016	0.147

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$

were not determined). Total blood calcium and phosphate were measured using absorption spectrophotometry. Serum osteocalcin was determined using an ECLIA method from Roche Diagnostics (Mannheim, Germany) and bone alkaline phosphatase (bALP) isoenzyme analysed using an IRMA kit from IMMUNOTECH (a Beckman Coulter company).

3.4. Statistics

The mean, standard deviation, median, maximum and minimum values were used for description of the investigated variables. To measure the dependence of two variables, while the subgroup of chosen variables was held constant, the partial correlation coefficient was used. Normality was tested by the Shapiro-Wilk's W statistic. All significant tests were two-sided and $P < 0.05$ as regarded as significant. Statistical software (SYSTAT Software, Inc., Chicago, IL, USA) was used for calculations.

4. Results

Table 1 shows the mean values (± SD) of all analysed parameters, including anthropometric data and the stage of puberty (Tanner 1-4).

Table 2 shows the unadjusted partial correlations between bone and body composition, muscle force, physical activity, and hormones. The simple correlations showed strong positive associations between either BMC or BMD L_1-L_4 and serum phosphate, estradiol, IGF-I, leptin, lean and fat volumes, and muscle force ($P < 0.001$). Positive correlations were observed between BMD L_1-L_4 and serum phosphate ($P < 0.01$), IGF-I ($P < 0.01$), estradiol ($P < 0.001$), leptin ($P < 0.01$), fat and lean masses ($P < 0.001$ for both the latter indices), and muscle force ($P < 0.001$) after removing linear effects of height, Tanner stage, and physical activity.

Table 3 indicates partial correlations of osteocalcin, bALP, BMC total, BMD L_1-L_4 and BMD Z-score with calcium, phosphate, serum and urine hormones, soft tissues, and neonatal parameters. 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 L_1-L_4 (g/cm^2) and BMD (Z-score) ($P < 0.001$, $P < 0.01$ and $P < 0.001$). A positive association was observed between leptin and the BMC and BMD Z-score ($P < 0.05$ for both bone parameters). Positive relationships were observed between serum osteocalcin and serum phosphate, 25 (OH) vitamin D and urine melatonin ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively). Similarly a positive correlation was seen between urine serotonin and bALP ($P < 0.05$). However, none of these hormonal parameters was correlated with BMC or BMD L_1-L_4. No correlations were observed between bone parameters and serum adiponectin. Partial correlations between lean mass or muscle force and hormone indices, after removing linear effects of height, Tanner stage and physical activity, are depicted in *Table 4*, which shows positive associations between lean volume or muscle force and serum IGF-I ($P < 0.01$ and $P < 0.01$, respectively) and between lean volume (but not muscle force) and serum leptin ($P < 0.05$). No as-

Table 3. Partial Correlations of Osteocalcin, bALP, BMC Total, BMD with Calcium, Phosphate, Serum and Urine Hormones, Soft Tissues, and Neonatal Parameters after Removing the Linear Effects of Height, Tanner Stage, and Physical Activity

	Osteocalcin	bALP	BMC Total	BMD L1-L4	BMD Z-Score
Ca	0.112	-0.061	0.022	0.004	-0.018
P	0.299 [b]	-0,028	-0.032	-0.093	-0.017
25(OH)D	0.252 [a]	-0.089	0.015	-0.001	0.028
PTH	0.227 [a]	0.184	-0.096	-0.111	-0.122
IGF-I	0.174	-0.007	0.090	0.074	0.131
IGF-I/IGFBP3	-0.069	-0.152	0.148	0.103	0.060
Estradiol	-0.037	-0.303 [b]	0.142	0.143	-0.096
Adiponectin	-0.018	0.107	0.211	-0.189	-0.252
Leptin	-0.214	0.115	0.303 [a]	0.217	0.275 [a]
Melatonin	0.313 [b]	-0.122	-0.138	-0.130	-0.242
Serotonin	0.168	0.234 [a]	-0.232	-0.245	-0.052
Fat mass	-0.385 [b]	-0.039	0.564 [c]	0.377 [b]	0.482 [c]
Lean mass	-0.100	-0.004	0.666 [c]	0.188	0.551 [c]
Muscle force	-0.046	0.001	0.349 [b]	0.188	0.242
Gestation	0.125	0.120	0.196	0.194	0.190
Breast feeding	-0.029	-0.002	0.011	-0.035	-0.061

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$

Table 4. Partial Correlations of Lean Mass and Muscle Force with Calcium, Phosphate, and Serum and Urine Hormones after Removing the Effects of Height, Tanner Stage, and Physical Activity.

	Lean Mass	Muscle Force
Ca	-0.024	-0.013
P	0.163	-0.021
25(OH)D	0.009	-0.212
1,25(OH)2D3	0.049	-0.050
PTH	0.118	0.034
IGF-I	0.363 [b]	0.261 [b]
IGFII/IGFBP3	0.302	0.308
Estradiol	0.043	-0.145
Adiponectin	-0.120	-0.228 [a]
Leptin	0.311 [a]	0.105
Melatonin	-0.046	0.039
Serotonin	-0,067	-0.169

[a] $P < 0.05$, [b] $P < 0.01$

sociations were found between the neonatal parameters and BMC or BMD.

5. Discussion

The study confirmed a positive association between pubertal bone and fat, or its hormone leptin, which is in agreement with the study on pubertal girls of Rhie *et al.* (19). Thus, both these studies support the hypothesis that the direct stimulating effect of leptin on developing bone mass prevails over its inhibitory effect mediated through central mechanisms (10).

In our girls the positive relationship was also found between leptin and lean mass values, which corresponds with the interesting results obtained by Olmedillas *et al.,* who found up-regulation of leptin receptors in the hypertrophic biceps of professional tennis players [24]. These data allow us to hypothesize that the positive effect of leptin on bone is partially mediated by muscle. It is generally accepted that muscle function is positively influenced by vitamin D. Ward *et al.* (9) demonstrated a close positive correlation between 25(OH) vitamin D levels and both muscle strength and high jump results in adolescent girls, with a corresponding negative relationship between PTH levels and these parameters. Moreover, positive correlations between 25 (OH) vitamin D and vertebral BMD and trochanter BMC has been documented in the study performed by El-Hajj Fuleihan *et al.* (20). In our study, however, we were unable to demonstrate any association between serum 25 (OH) vitamin D or PTH and either lean or bone mass. The reason for the lack of an obvious relationship between 25 (OH) vitamin D levels and muscle function is not clear from our study, but could be partially explained by the fact that girls in the El-Hajj Fuleihan study were undernourished and the girls studied by Ward were younger than our participants. The start of female puberty is induced by an increase in sexual steroid production. The osteotropic effect of estrogens (or androgens) is direct, but partly mediated by IGF-I provided that the nutritional status and physical activity levels are appropriate (21). In our study relationships between bone parameters and estradiol or IGF-I levels could be showed in unadjusted analyses. However, these associations lost statistical significance after eliminating the effects of age, Tanner stage, and physical activity level. Since IGF-I production and bone response to the hormone depend partly on physical activity (7), the lack of an adjusted-association between serum IGF-I levels and bone mass could be explained by relatively low physical activity levels in our girls. On the other hand, the effect of protein-caloric malnutrition could be excluded for the majority of our participants. Serotonin has a bimodal influence on target tissues, including bones, depending on the source of its synthesis (duodenum or central nervous system). Serotonin produced by enterochromafin cells (95% of total production) inhibits bone formation, whereas serotonin produced in the central nervous system has the opposite effect (22). The inconclusiveness of association between serotonin levels and bone parameters in our girls could be explained by the sum of impacts from both of these serotonin pools. The concept of osteoanabolic effects for melatonin is supported in the present study by a proven positive association between nocturnal melatonin excretion levels and osteocalcin levels. However, we did not observe any relationships be-

tween serum melatonin and either BMC or BMD, which could be, in part, explained by the physiological decline of this hormone during puberty. Thus, melatonin does not seem to play a key role in the development of bones in human females (23).

As expected, the study demonstrated significant positive associations between bone mass and serum leptin levels in peripubertal girls. However, the study yielded some surprising results. It failed to demonstrate any relationship between bone parameters and IGF-I levels, even though IGF-I is positively correlated with muscle mass and muscle force. Similarly, the association between muscle parameters and 25 (OH) vitamin D levels was inconclusive in girls. Nevertheless, the study allows us to theorize that a delay in puberty and the deficient development of muscle or fat slows bone mass development via its direct effects on bones, independently of the vitamin D status. Positive associations between the factors of lean tissue mass, muscle force, and physical activity on the one hand and BMC or BMD on the other hand are compatible with the muscle-bone unit concept. Continuous monitoring of sexual development, together with tissue composition and hormone parameters (leptin and IGF-I), might help in the early identification of girls with a later high risk of fractures due to inadequate development of peak bone mass.

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