Pediatric Endocrinology Diabetes and Metabolism
Endokrynologia, Diabetologia i Choroby Przemiany Materii Wieku Rozwojowego

Original Paper | Praca oryginalna
Pediatr Endocrinol Diabetes Metab 2015;21,4:154-161
DOI: 10.18544/PEDM-21.04.0037

Bone turnover markers in the obese children – relation to gender, body composition and leptin level

Markery obrotu kostnego u otyłych dzieci – związek z płcią, składem masy ciała i stężeniem leptyny

[1]Paweł Matusik, [2]Magdalena Olszanecka-Glinianowicz, [3]Jerzy Chudek, [1]Ewa Małecka-Tendera

[1]Department of Pediatrics and Pediatric Endocrinology, School of Medicine in Katowice, Medical University of Silesia, Katowice
[2]Health Promotion and Obesity Management Unit, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland
[3]Pathophysiology Unit, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

Abstract

Introduction. Recently published data revealed that bone turnover is related to the body composition in pubertal children and may be impaired in obese adolescents. The aim of the study was to determine the relationship between bone turnover markers, body composition and leptin level in obese children. **Material and methods.** In 54 obese adolescents (25 boys and 29 girls) in the mean age of 13.96 ±2.78 years bone turnover markers – osteocalcin (OC), N-terminal telopeptide of type I collagen (NTx), OC/NTx ratio and leptin were determined. Anthropometric parameters expressed as BMI Z-score, WHR, W/HtR and body composition was evaluated by bioelectrical impedance analysis (BIA)such as fat mass (FAT), fat-free mass (FMM), predicted muscle mass (PMM) and total body water (TBW). The results were compared to the control group of 75 normal weight children (25 boys and 38 girls). **Results.** OC was significantly lower in obese children, particularly in obese girls ($p<0.05$ and $p<0.0001$ respectively). Bone turnover ratio (calculated as OC/NTx) was significantly lower in obese girls only ($p<0.01$). Significant negative correlation was found between the OC level and BMI Z-score in the whole studied population of children. OC and OC/NTx correlated significantly with all anthropometrical parameters only in girls. There was also a significant positive correlation between NTx and leptin in the entire group, being significantly higher in females ($p<0.05$ and $p<0.0001$ respectively). **Conclusions.** Bone turnover is related to the amount of fat mass and its hormonal activity. We can suspect that, in obese children, particularly in obese adolescent girls, impairment of bone turnover may be a risk factor for the lower bone mass and higher fracture risk in the future life.

Key words

obesity, children, osteocalcin, body composition, leptin

Streszczenie

Wstęp. Według aktualnych doniesień obrót kostny w okresie dojrzewania jest związany ze składem masy ciała, a u otyłych dzieci nadmierna masa tkanki tłuszczowej może mieć niekorzystny wpływ na masę kostną. **Celem pracy** była ocena zależności pomiędzy markerami obrotu kostnego, stanem odżywienia i stężeniem leptyny u dzieci otyłych. **Materiał i metodyka.** W grupie 54 otyłych dzieci (25 chłopców i 29 dziewcząt) w wieku 13,96±2,78 lat oznaczono stężenia osteokalcyny (OC) oraz N-końcowego telopeptydu kolagenu typu I (NTx). Ponadto wyznaczono wskaźnik OC/NTx i stężenie leptyny. Oceny antropometrycznej dokonano na podstawie BMI Z-score, wskaźników WHR i W/HtR oraz analizy parametrów składu ciała oznaczonych metodą bioimpedancji elektrycznej (BIA) (tkanka tłuszczowa – FAT, tkanka beztłuszczowa – FFM, przewidywana masa mięśniowa – PMM i całkowita zawartość wody – TBW). Grupę kontrolną stanowiło 75 zdrowych dzieci (38 chłopców i 37 dziewcząt) z prawidłową masą ciała. **Wyniki.** OC była znamiennie niższa w całej grupie dzieci otyłych, szczególnie w grupie otyłych dziewcząt (odpowiednio $p<0,05$ i $p<0,0001$). Obrót kostny wyrażony poprzez OC/NTx był znamiennie niższy tylko w grupie otyłych dziewcząt ($p<0,01$). Stwierdzono znamienną negatywną korelację pomiędzy OC i BMI Z-score u wszystkich badanych dzieci. Markery obrotu kostnego (OC i OC/NTx) korelowały znamiennie ze wszystkimi parametrami antropometrycznymi tylko u dziewcząt. Ponadto poziom NTx korelował dodatnio ze stężeniem leptyny zarówno w całej populacji, jak i w grupie dziewcząt (odpowiednio $p<0,05$ i $p<0,0001$). **Wnioski.** Obrót kostny jest zależny zarówno od ilości tkanki tłuszczowej, jak i jej hormonalnej aktywności. Można przypuszczać, że zaburzony obrót kostny u otyłych dzieci,

Dr n. med. Paweł Matusik,
Katedra i Klinika Pediatrii i Endokrynologii Dziecięcej,
ul. Medyków 16; 40-752 Katowice;
e-mail: endocrine@wp.pl; pmatusik73@gmail.com;
tel.: +48322071654; fax.: +48322071653

Received: 2015-12-01.
Accepted: 2015-12-31.
Conflict of interests: none declared.

a szczególnie u otyłych dziewcząt w okresie dojrzewania, może negatywnie wpływać na jakość tkanki kostnej i wiązać się z większym ryzykiem złamań w późniejszym okresie życia.
Słowa kluczowe
otyłość, dzieci, osteokalcyna, skład masy ciała, leptyna

Introduction

Childhood obesity epidemic is nowadays the most important challenge for public health system worldwide, mainly in the developed countries [1–4]. Recently, there has been a growing concern that childhood obesity may negatively affect bone development [5–8]. However, some studies report no negative impact on growing skeleton in obese children [9,10]. Therefore determining whether excess adiposity is beneficial or detrimental to the bone quality in obese children is a scientific challenge. Recently published review by Paulis et al. showed that obesity and overweight are associated with a significant increase of musculoskeletal complaints in children including higher fractures rate [5]. Moreover study performed by Foley et al. showed that low bone mineral density is associated with high fat mass and a higher fractures risk [6].Cole et al [7] reported that fat mass was negatively correlated with volumetric bone mineral density in a group of 6 years old children.

Puberty is a period of marked changes in the human body composition and gender differences in adiposity, fat free mass and bone density become striking [11]. A limited number of studies reported inconsistent findings on an independent cross-talk between bone turnover intensity and anthropometrical parameters and adipose tissue activity, especially in the period of pubertal development [12–15]. With respect to the dramatic rise in obesity prevalence among children and adolescents worldwide, an understanding of the links between body composition and bone metabolism during the pubertal period seems to be very important to manage potentially adverse consequences for metabolic and skeletal health in adult life.

The aim of the present study was to determine the relationship between bone turnover markers, nutritional status and leptin levelin obese children compared to the lean controls matched for age.

Materials and methods

Studied population

The study Group (SG) comprised 54 obese adolescents(25 boys and 29 girls) in the mean age of 13.96 ±2.78 years. They were consecutively recruited for the study from the patients referred to Outpatients Obesity Department. Children with syndromic obesity and endocrine disorders associated with obesity were excluded. Other exclusion criteria were factors that could influence bone turnover like chronic diseases (i.e. asthma), several fractures history, and medications (i.e. glucocorticoids, vitamin D, calcium, other vitamins). The control group (CG) comprised 74healthy children (25 boys and 38 girls) matched for age, sex and pubertal status. They were all healthy, of normal weight and did not take any medications.

Anthropometric measurements

Standing height was measured by a wall-mounted Harpender Stadiometer to the nearest 0.1 cm and weight by an electronic scale with readings accurate to 0.1 kg, measured in children in their underwear.Body mass index (BMI) was calculated using the standard formula (kilograms per meter squared). BMI z-scores were derived using WHO AnthroPlus, version 1.0.4 (based on World Health Organization growth references) [16]. Obesity was defined as BMI at or above the 95th percentile for age and sex, using the WHO charts [16]. Waist and hip circumferences were measured midway between the lower rib margin and the iliac crest in the standing position and Waist/Hip Ratio (WHR) and Waist/Height Ratio (W/HtR) were calculated. For the pubertal stage evaluation standard Tanner criteria were used [17].

Body composition analysis

Body composition parameters: fat mass (FAT), fat-free mass (FFM), total body water (TBW) and predicted muscle mass (PMM) were assessed (in kilograms [kg] or as percentage of body weight [%]) based on bioelectrical impedance using segmental body composition analyzer (BC-418MA Tanita Europe BV, Hoofddorp, Nederlands).

Biochemical analysis

Venous blood samples were drawn from antecubital vein in the morning in the supine position after the overnight fasting and collectedinto vacutainer tubes. After centrifugation at 1500 x g at 4°C for 5 min, serum was collected and transferred in Eppendorf™ tubes, then immediately frozen and stored at -80°C until analysis. Competitive-inhibition enzyme-linked immunosorbent assay (ELISA) was used to evaluate amino terminal collagen cross-links (NTx) in serum (Osteomark NTx Serum).Quantitative sandwich enzyme immunoassay technique was used for the measurement of osteocalcin (OC) (MicroVue Osteocalcin EIA kit, Quidel, San Diego, USA) and leptin (TECOmedical AG, Swissach, Switzerland) . All samples were tested in duplicate.

Ethical considerations

The study was approved by the Ethics Committee of the Medical University of Silesia. All participants and/or their caregivers gave informed consent. Patient rights were also approved according to the Helsinki Declaration.

Statistical analysis

The normal distribution of all the variables was assessed by the Kolmogorow-Smirnov test. The following variables were

not normally distributed, and were log transformed to achieve near-normal distributions: leptin, osteocalcin, and NTx. Baseline comparisons of categorical variables were performed by χ^2 test. Differences in continuous variables between the studied groups were assessed by Student's t-test and were adjusted for sex. Correlations between continues parametrical (or log transformed) variables were based on linear Pearson's correlation coefficient. All statistical analyses were made with the Statistica™ 12 PL software and p value less than 0.05 was considered statistically significant.

Results

Baseline characteristics

Baseline characteristics, differences of either anthropometric measurements or bone turnover markers and leptin of all studied children are reported in table I. Subjects in the study and control groups were comparable with respect to age, gender and Tanner stage distribution. As expected, there were strong significant differences between the groups concerning all anthropometrical variables and the leptin level. Moreover,

Table I. Characteristics and comparison of the anthropometric measurements, leptin level and bone turnover markers among the obese and lean children

Tabela I. *Charakterystyka porównawcza parametrów antropometrycznych, stężeń leptyny i markerów obrotu kostnego pomiędzy grupą dzieci otyłych i szczupłych*

	Study group (Obese) n = 54	Control group (Lean) n = 75	P value
Age [years]	13.21 ± 2.8	13.08 ± 2.4	NS
Sex [M/F]	25/29	38/37	NS
Tanner stage [II/III/IV]	15/29/10	21/38/16	NS
BMI [kg/m²]	30.84 ± 4.34	19.61 ± 2.78	< 0.0000001
Boys	30.42 ± 4.15	19.51 ± 2.54	< 0.0000001
Girls	30.79 ± 4.71	19.44 ± 2.84	< 0.0000001
BMI Z score [SD]	3.00 ± 1.20	0.30 ± 0.86	< 0.0000001
Boys	3.28 ± 1.62	0.43 ± 0.77	< 0.0000001
Girls	2.68 ± 0.57	0.10 ± 0.88	< 0.0000001
WHR	0.94 ± 0.07	0.81 ± 0.06	< 0.0000001
Boys	0.96 ± 0.07	0.84 ± 0.07	< 0.0000001
Girls	0.92 ± 0.07	0.75 ± 0.04	< 0.0000001
W/HtR	0.61 ± 0.06	0.43 ± 0.04	< 0.0000001
FAT [%]	36.60 ± 6.78	21.46 ± 4.80	< 0.0000001
FFM [%]	63.41 ± 6.78	78.56 ± 4.79	< 0.0000001
PMM [%]	60.42 ± 6.56	75.05 ± 4.80	< 0.0000001
Leptin [ng/ml]	22.04 ± 18.82	6.98 ± 5.59	< 0.0000001
OC [ng/ml]	11.23 ± 8.71	15.03 ± 9.97	< 0.05
NTx [nM/BCE]	50.46 ± 60.86	49.21 ± 44.88	NS
OC/NTx	0.35 ± 0.26	0.39 ± 0.31	NS

Data are expressed as mean ± standard deviation and compared using student's t-test and Chi square test

Abbreviations: BMI – body mass index, BMR – basal metabolic rate, FAT – fat mass, FFM – fat free mass, NTx – amino-terminal collagen crosslinks, OC – osteocalcin, PMM – predicted muscle mass, WHR – waist/hip ratio, W/HtR – waist/height ratio, nM/BCE – nmol/Bone Collagen Equivalents

the OC level was significantly lower in the obese adolescents ($p<0.05$). Bone turnover ratio (OC/NTx) was also lower in obese group but the difference was not statistically significant.

Bone turnover markers and leptin in relation to the gender and anthropometrical status

The results are presented in table II. Bone formation marker (OC) and bone turnover ratio (OC/NTx) were significantly higher in girls ($p<0.05$). It was expected due to more advanced pubertal development in girls with the same age. However, those physiological differences were present only in the lean control group ($p<0.001$ and $p<0.01$ respectively), whereas bone turnover markers in obese girls did not differ compared to obese boys in the same age. Moreover, bone turnover markers (OC and OC/NTx) were significantly lower in obese girls vs. lean controls ($p<0.0001$ and $p<0.01$ respectively). As expected, the leptin level was significantly higher in the obese group than in the lean subjects ($p<0.000001$ for boys and $p<0.01$ for girls respectively). However, in all studied children, there was no physiological difference between girls and boys regarding to leptin, which was due to more than five times higher leptin level in obese boys than in the lean control group ($p<0.000001$). The expected gender physiological differences regarding the leptin level was found only within the control group ($p<0.000001$). No significant difference was found in the NTx level between obese and lean subjects in both sexes.

Correlation between bone turnover markers, and nutritional status and leptin level

All significant correlations found within the parameters in the entire studied population, obese study group and all girls are reported in table III. A significant negative correlation was found between the OC level and BMI Z-score in all studied population ($p<0.05$). OC and OC/NTx correlated significantly with all anthropometrical parameters only in girls. Correlations between the OC and body composition parameters assessed by BIA reached highest significance ($p<0.00001$). There was also significant positive correlation between bone resorption marker (NTx) and leptin level in the entire group as well as in girls ($p<0.05$ and $p<0.0001$ respectively). There were no significant correlations between anthropometrical status markers, leptin and bone turnover in lean subgroup an in boys (table IV).

Discussion

Serum OC levels are used to evaluate bone metabolism, as a bone formation marker. However, an increasing amount of data has emerged to support extra-skeletal effects of OC [18,19]. In our study, the OC level was significantly lower in the obese group. However, after the adjustment to gender, the significance was present only in girls. Also, bone turnover ratio (OC/NTx) was significantly lower only in obese girls. Similar observation was made by Dimitri at al [12] who showed reduced bone formation relative to resorption in group of 103 obese children. In our study, the OC level correlated significantly with BMI

Table II. Comparison of the bone turnover markers and leptin level with respect to anthropometrical status and gender
Tabela II. *Porównanie markerów obrotu kostnego i stężenia leptyny w zależności od stanu odżywienia i płci*

	All studied population n = 129		P value	Study group (Obese) n = 54		P value	Control group (Lean) n = 75		P value	Boys n = 63		P value	Girls n = 66		P value
	Boys N = 63	Girls N = 66		Boys N = 25	Girls N = 29		Boys N = 38	Girls N = 37		Obese N = 25	Lean N = 38		Obese N= 29	Lean N = 37	
OC [ng/ml]	11.72 ± 8.83	15.08 ± 10.09	<0.05	12.86 ± 9.58	9.82 ± 7.77	NS	10.97 ± 8.35	19.20 ± 9.87	<0.001	12.86 ± 9.58	10.97 ± 8.35	NS	9.82 ± 7.77	19.20 ± 9.87	<0.0001
NTx [nM/BCE]	57.25 ± 53.65	40.53 ± 50.10	NS	57.22 ± 56.31	44.51 ± 65.16	NS	57.26 ± 52.74	35.80 ± 22.55	NS	57.22 ± 56.31	57.26 ± 52.74	NS	44.51 ± 65.16	35.80 ± 22.55	NS
OC/NTx	0.32 ± 0.24	0.44 ± 0.33	<0.05	0.35 ± 0.25	0.36 ± 0.28	NS	0.30 ± 0.24	0.55 ± 0.36	<0.01	0.35 ± 0.25	0.30 ± 0.24	NS	0.36 ± 0.28	0.55 ± 0.36	<0.01
Leptin [ng/ml]	10.16 ± 13.25	14.52 ± 14.49	NS	21.36 ± 16.87	22.74 ± 21.10	NS	3.97 ± 3.27	10.07 ± 5.81	<0.000001	21.36 ± 16.87	3.97 ± 3.27	<0.000001	22.74 ± 21.10	10.07 ± 5.81	<0.01

Data are expressed as mean ± standard deviation and compared using student's t-test

Table III. Significant correlations between nutritional status parameters, leptin level vs. bone turnover markers
Tabela III. *Znamienne korelacje pomiędzy parametrami antropometrycznymi, stężeniem leptyny a markerami obrotu kostnego*

All studied population (n = 129)		
	OC [ng/ml]	
	Pearson's correlation	*Significance*
BMI z-score [SD]	r = -0.194	p < 0.05
Study group (n = 54)		
	NTx [nM/BCE]	
	Pearson's correlation	*Significance*
Leptin [ng/ml]	r = 0.360	p < 0.05
Girls (n = 66)		
	OC [ng/ml]	
	Pearson's correlation	*Significance*
BMI z-score [SD]	r = -0.495	p < 0.0001
W/HtR	r = -0.332	p < 0.05
WHR	r = -0.343	p < 0.05
FAT [%]	r = -0.575	p < 0.000001
FFM [%]	r = 0.576	p < 0.000001
PMM [%]	r = 0.575	p < 0.000001
BMR [kJ/kg]	r = 0.619	p < 0.000001
	OC/NTx	
	Pearson's correlation	*Significance*
BMI z-score [SD]	r = -0.343	p < 0.05
W/HtR	r = -0.333	p < 0.05
FAT [%]	r = -0.322	p < 0.05
FFM [%]	r = 0.323	p < 0.05
PMM [%]	r = 0.320	p < 0.05
	NTx [nM/BCE]	
	Pearson's correlation	*Significance*
Leptin [ng/ml]	r = 0.608	p < 0.0001

Table IV. Correlations between nutritional status parameters, leptin level vs. bone turnover markers in boys
Tabela IV. *Korelacje pomiędzy parametrami antropometrycznymi, stężeniem leptyny a markerami obrotu kostnegou chłopców*

Boys (n = 63)		
	OC [ng/ml]	
	Pearson's correlation	***Significance***
BMI z-score [SD]	r = 0.116	NS
W/HtR	r = 0.003	NS
WHR	r = 0.082	NS
FAT [%]	r = 0.187	NS
FFM [%]	r = -0.186	NS
PMM [%]	r = -0.189	NS
BMR [kJ/kg]	r = -0.167	NS
	OC/NTx	
	Pearson's correlation	***Significance***
BMI z-score [SD]	r = 0.030	NS
W/HtR	r = 0.046	NS
FAT [%]	r = 0.029	NS
FFM [%]	r = -0.030	NS
PMM [%]	r = -0.031	NS
	NTx [nM/BCE]	
	Pearson's correlation	***Significance***
Leptin [ng/ml]	r = -0.025	NS

Z-score in the entire studied population, whereas in girls the OC and OC/NTx were significantly related to all anthropometrical measurements and body composition parameters. Similar relation between the OC and BMI was found by Dubnov-Raz et al. in the group 160 of healthy adolescent girls [15]. The other study showed the inverse significant relation between the OC and adiposity (BMI and fat mass) or leptin level in the group of adolescent boys [14].Similar data was published by Reinehr et al [20]. Osteocalcin levels were significantly lower in obese children compared to a non-obese control group. In the otherstudy osteocalcin levels were found to be inversely correlated with fat mass, fat percentage and BMI in a group of 106 children aged 11–14 years [21].

In our study, the leptin level was significantly higher in obese children but there was also a significant positive correlation between bone resorption marker (NTx) and leptin in the entire group and the higher significance was showed in girls. The recent study, performed by Dimitri et al. [22], showed that childhood obesity alters the radial and tibial microstructure assessed by high resolution peripheral quantitative computed tomography (HR-pQCT). Moreover, this process may be mediated by leptin which was inversely related to the radial cortical porosity and tibial trabecular thickness. In accordance with our results were the data published by the same authors [12] who showed significant positive correlation between leptin and bone resorption marker such as CTx in obese children [12]. Support for leptin acting as a key hormone disturbing bone development in obese children also comes from studies in children with profound changes in body composition. Data based on studies performed in children with congenital leptin deficiency showed a normal age and sex related bone mineral content and density despite hypogonadism and hyperparathyroidism coincidence [23,24]. It may suggest that severe leptin deficiency may have a protective value for bone in those subjects. Alteration in skeletal microarchitecture in adolescence result in transient skeletal weakness in mid-puberty may coincide with the period of peak fracture incidence [25]. The over-representation of overweight and obese children in fractures stud-

ies suggests that excess fat in children may alter bone mineral density and bone quality that increased this risk [8,22,26,27]. Our study suggests that the association between bone turnover markers and the leptin level in entire studied population and especially in girls may be dependent on anthropometrical parameters and body composition. The similar findings were described by Lucey et al. which revealed the significant correlation between the leptin level and urinary NTx in the group of 268 young women [28]. However, the role of leptin in the cross-talk between fat and bone still need to be extensively studied, because the recent data showed either negative (via hypothalamic action on the sympathetic nervous system) or positive (via mesenchymal cells differentiation towards osteoblasts) impact [29-31].

The primary limitation of our study was the different puberty stage distribution within the group of boys and girls. However, as obese children enter puberty earlier, there were no significant differences for the pubertal stages within either boys or girls subgroups. The data based on Polish population demonstrated that the peak values of the OC level occurred much earlier in pubertal girls than in boys (between 9–13 and 10–15 years respectively) [32]. Moreover, OC seems to be a useful parameter to assess the pubertal growth spurt [13, 33]. Therefore, further studies based on the larger groups of mid-pubertal obese boys are needed regarding bone turnover intensity in relation to the adipose tissue overload and adipokines production.

Other limitation of our study was the usage of bioelectrical impedance analysis (BIA) which is an indirect method of body composition assessment. Areal BMD (aBMD) measured by dual-energy X-ray absorptiometry (DXA) is currently the gold standard not only for the diagnosis of osteoporosis but also for the body composition evaluation. However, a good correlation between BIA and DXA has been reported in estimating adiposity in the different groups of patients [34,35]. BIA is a relatively simple, quick, non-invasive and readily accessible compared to other methods, such as quantitative computed tomography (qCT) or DXA. A more widespread use of DXA in children is limited mainly by its costs and exposure to X-ray radiation. The process of BIA validation resultedin thedevelopment ofstandards andcentile charts forhealthy children [36]. BIA is more accurate than skinfold thickness and BMI when compared with a reference method [37], but accuracy may be lower for the severely obese children [38] and pediatric population with diabetes type 1 [39]. Therefore, BIA seems to be a useful noninvasive tool for the body composition assessment in pediatric population.

Conclusions

In conclusion, our findings clearly demonstrate that bone turnover may be altered in the obese children (especially in girls) and pathogenic factor which can be involved in that mechanism may be either adipose tissue overload as well as its hormonal activity expressed as leptin excess. Moreover, our data suggest that the impairment of bone turnover ratio in obese pubertal girls may be a risk factor for the lower bone strength and higher fracture risk in the pubertal period and the insufficient peak bone mass accrual leading to the earlier osteoporosis in the future.

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