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ORIGINAL ARTICLE

The impact of excess body fat on bone remodeling in adolescents

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Abstract
Summary The impact of excess body fat on bone remodeling was evaluated in overweight, obese, and extremely obese adolescents. In adolescents with excess weight, it was observed that the higher the bone mineral content and bone mineral density values, the lower the levels of the biomarkers. Nutritional imbalances by excess had a negative effect on bone formation in this stage of life.
Introduction The aim of this study was to investigate the impact of excess body fat on bone remodeling in adolescents.
Methods Body weight, height, and body mass index were determined in 391 adolescents classified as normal weight, overweight, obese, and extremely obese. Bone age was obtained and bone mineral content and bone mineral density were evaluated in the lumbar spine, proximal femur, and total and subtotal body. Blood samples were collected for evaluation of the following bone biomarkers: osteocalcin, bone alkaline phosphatase (BAP), and serum carboxy-terminal telopeptide (S-CTx). The data were analyzed according to nutritional status and age.
Results In girls with excess weight, the biomarkers were higher in the 10 to 13-year age group and no significant differences were observed between groups according to nutritional status. In boys, the levels were higher in those aged 13 to 15 years. According to nutritional status, significant differences were only observed in mean S-CTx for the age groups of 10–15 years, with higher levels between overweight and obese adolescents aged 10–12 years and between obese and extremely obese adolescents aged 13–15 years. In girls, significant negative correlations were observed between lean mass, fat mass, and fat percentage and each of the three bone markers studied. There was no correlation between lean mass

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or fat mass and the three biomarkers in boys. The biomarker trends demonstrated across the age groups follow the age trends for growth velocity.
Conclusions The higher the fat percentage and fat mass in girls, the lower the levels of the biomarkers, indicating that excess body fat has a negative effect on the evolution of these markers during adolescence.

Keywords Adolescence · Bone mineral content · Bone mineral density · Bone turnover · Obesity

Introduction

Osteoporosis and obesity are common chronic diseases that are associated with negative health consequences. The first two decades of life have been indicated as critical periods for the prevention of osteoporosis since childhood and puberty are phases of exponential bone mass development. Furthermore, bone tissue has been recognized as an endocrine organ and a complex link with adipose tissue has been proposed since adipocytes and osteoblasts originate from the same cell line [1]. It therefore remains necessary to elucidate the impact of excess body fat, already present during those years, on bone mass accrual [2].

An increase in bone mass of approximately 8.5% per year occurs in adolescents exactly during this period of intense physical changes that comprises the growth spurt. This requires that a sequence of events involving bone formation and resorption starts in childhood and continues during adolescence. Biochemical markers of bone metabolism are released during the processes of bone turnover by the physiologically coupled action of osteoclasts and osteoblasts [3]. Studies using animal models and recent human studies have demonstrated that osteocalcin, a biologically active marker associated with glucose metabolism, influences the release of and sensitivity and resistance to insulin [4, 5], with high concentrations being associated with low insulin resistance.

Furthermore, adipose tissue plays an important role in bone metabolism because of the production of the so-called adipokines. Some adipokines exert a positive effect on bone formation by promoting the maturation of osteoblasts and others are predictors of orthopedic fractures [1, 6], while some adipokines can inhibit or stimulate the activation of osteoblasts and osteoclasts through different mechanisms [7, 8].

In adolescents with excess weight, the effects of obesity on bone mass and its relationship with biochemical markers of bone remodeling are not fully elucidated. Dimitri, Wales, and Bishop [9] evaluated 103 children aged 5 to 16 years divided into two groups (extremely obese/obese: $n = 52$ and normal weight: $n = 51$). The authors found that fractures were more common in the excess weight group, suggesting lower bone mass and thus thinner bones in these patients with excess fat mass. Another recent study demonstrated a reduction in bone mass, evaluated by bone densitometry using dual energy X-ray absorptiometry (DXA), in extremely obese adolescents when compared to overweight adolescents [10].

Within this context, the objective of the present study was to evaluate the evolution of some biomarkers of bone formation and resorption in adolescent girls and boys with excess weight resulting from the accumulation of fat mass, who were classified as overweight, obese, and extremely obese, in order to elucidate the evolution of bone mass in response to this severe nutritional problem.

Materials and methods

This is a cross-sectional study involving 391 adolescents aged 10–19 years. Data were collected from March 2010 to March 2012. The sample was selected based on the availability of care for new cases at the Adolescent Outpatient Clinic of the University Hospital, Botucatu Medical School (SP, Brazil). The adolescents were invited to participate in the study at the time of their visit to the outpatient clinic and an informed consent form was signed by the adolescents and by their parents or legal guardians. The study was approved by the Research Ethics Committee of the Botucatu Medical School (UNESP, OF.190/2009).

Weight (kg) and height (m) were obtained as described previously [11] and the body mass index (BMI, kg/m^2) was subsequently calculated and used to classify nutritional status. Adolescents were classified as normal weight (between the 5th and 85th percentiles), overweight (≥85th and <95th percentiles), obese (≥95th percentile), and extremely obese (>99th percentile) according to BMI curves for age and gender [12–14]. The adolescents were non-smokers and non-drinkers. Furthermore, the inclusion criteria required that the adolescents had not participated in regular sports activities during the 6 months prior to the study, except for the physical education classes at school, twice a week for 50 min each class, which corresponds to a low level of physical activity [15].

In adolescents, chronological age (CA) is often considered to be imprecise and the use of bone age (BA) and pattern of pubertal events is therefore a sensitive alternative for comparison between them. The use of BA in the present study is justified based on a previous study, which demonstrated a correlation of 0.93 between BA and pubertal events [16].

For the analyses, knowing the BA at which the highest growth velocity for each sex occurs, female adolescents were grouped according to BA into four groups: BA1, 10 to 11 years; BA2, 12 to 13 years; BA3, 14 to 15 years, and BA4, 16 to 19 years. Male adolescents were grouped into three age groups: BA1, 10 to 12 years; BA2, 13 to 15 years, and BA3, 16 to 19 years. To adjust for chronological age

(CA), all adolescents were subdivided into three groups: 10 to <13 years; 13 to <16 years, and >16 years.

Exclusion criteria were (a) a history of prematurity; (b) weight higher than 100 kg (as this value exceeds equipment's manufacturer recommendations for bone densitometry measures); (c) long-term therapy with corticosteroids; (d) use of supplemental calcium and/or iron in the last 12 months prior to data collection; (e) presence of congenital or acquired bone disease, gastrointestinal disease, a history of renal disease, endocrine disorders, precocious or delayed puberty; (f) chronic medication use; (g) use of hormonal contraceptives; and (h) being or having been pregnant. Dietary exclusion criteria were the consumption of an exclusive high-fiber vegetarian diet with a fiber content above age-appropriate recommendations (>26 g/day for female adolescents or >31 g/day for male adolescents (9–13 years) or >38 g/day (males and females aged 14–16 years) [17], caffeine intake >300 mg/day (>3 cups of coffee per day) [18–20], and lack of daily consumption of dairy products.

To evaluate skeletal maturation, BA was obtained by the Greulich-Pyle method [21], in which hand and wrist radiographs were compared to the atlas. Bone age was analyzed by an expert radiologist who was unaware to which weight group the participant belonged. The adolescents were then submitted to bone densitometry by DXA (Hologic QDR 4500 Discovery A, Hologic, Inc., Bedford, MA). The bone mass results were analyzed with appropriate pediatric software and the bone mineral content (BMC) results are expressed as gram and as density in g/cm^2. Measurements were taken in the L1–L4 lumbar spinal region and total proximal femur, including the femoral neck, trochanteric and intertrochanteric regions, subtotal body (whole body less head), and whole body (to obtain total BMC, BMD, and whole-body composition) [22].

Blood samples were collected by venous puncture and centrifuged for 15 min at 1500*g* for the separation of serum. The serum samples were stored at −70 °C until the time of analysis of the biomarkers (bone alkaline phosphatase (BAP), osteocalcin and carboxy-terminal telopeptide (S-CTx)). BAP and intact osteocalcin were measured using an assay from Metra™ Biosystems (San Diego, CA, USA), with intra and interassay coefficients of variation of 8 and 7.6%, respectively. S-CTx was quantified by electroimmunochemiluminescence (ECLIA) using the Elecsys beta-Cross Laps serum assay in an automated Elecsys device (Roche™, Indianapolis, IN, USA). The interassay coefficient of variation was 5%.

Statistical analysis

The quantitative variables were submitted to descriptive analysis and are reported as the mean, standard deviation and median. Quantitative variables showing a normal distribution were compared between groups by analysis of variance (ANOVA) with simple classification, followed by the Tukey test. For the remaining analyses, interference of BA was verified in the groups to avoid that a possible difference found would not only be a casual effect.

The variables BAP, osteocalcin, and S-CTx showed an asymmetrical distribution. The GENMOD procedure of the SAS for Windows v.9.2 program was used for the comparison between groups stratified by sex, applying a generalized linear model with gamma distribution. Multiple comparisons were performed by the same procedure using the DIFF option of the same program (Wald test).

The associations between the bone biomarkers (BAP, osteocalcin, and S-CTx) and lean mass (LM), fat mass (FM), body fat percentage (BF%), BMI, BMC, and BMD were evaluated using Pearson's correlation test. The same associations were obtained stratifying by sex. In all tests, the level of significance was set at 5% or the corresponding *p* value was used.

Results

Among the 391 adolescents participating in the study, 158 (40.4%) were classified based on BMI as normal weight, 82 (20.97%) as overweight, 105 (26.85%) as obese, and 46 (11.78%) as extremely obese; 208 (53.19%) were females and 183 (46.81%) were males.

The mean CA and BA of the adolescents were calculated according to the classification of nutritional status based on BMI. The mean CA or BA did not differ significantly between weight groups in either girls or boys. Advancement of mean BA over mean CA was observed in all weight groups according to sex (Table 1).

In both genders, significant differences in weight and BMI were observed between all nutritional groups adjusted for CA and BA. There was no significant difference in height when adjusted for CA or BA. In most adolescent boys and girls, the mean values of LM, FM, and BF% increased from the normal weight to the extremely obese state and the differences were significant ($p < 0.01$). With respect to LM, no significant differences were observed between obese and extremely obese adolescents, but the amount of fat in gram and BF% differed significantly between all weight groups in boys and girls ($p < 0.01$) (Table 2).

Table 3 shows the analysis of the bone biomarkers (BAP, osteocalcin, and S-CTx) in male and female adolescents with excess weight according to BA. In girls, the three biomarkers showed a similar profile, with higher values in the 10 to 12-year and 12 to 13-year age groups, which correspond to the period of maximum acceleration of growth when the peak height velocity (PHV) occurs. In boys, the mean levels of

Table 1 Chronological age and bone age of male and female adolescents according to body mass index

	BMI				
	Normal weight	Overweight	Obese	Extremely obese	*P*
Female CA (years)	(*n* = 72)	(*n* = 53)	(*n* = 62)	(*n* = 21)	
	13.64[a] ± 2.76	14.42[a] ± 2.41	13.66[a] ± 2.44	13.49[a] ± 1.65	0.050
Male CA (years)	(*n* = 86)	(*n* = 29)	(*n* = 43)	(*n* = 25)	
	13.48[a] ± 2.53	14.05[a] ± 2.01	13.76[a] ± 1.92	13.73[a] ± 1.92	0.753
Female BA (years)	(*n* = 72)	(*n* = 53)	(*n* = 62)	(*n* = 21)	
	14.39[a] ± 2.61	14.94[a] ± 2.41	14.53[a] ± 2.26	14.40[a] ± 2.17	0.794
Male BA (years)	(*n* = 86)	(*n* = 29)	(*n* = 43)	(*n* = 25)	
	13.98[a] ± 2.68	14.74[a] ± 2.51	14.41[a] ± 1.87	14.56[a] ± 1.26	0.550

Same letters indicate no significant differences among groups (normal weight, overweight, obese and extremely obese). ANOVA followed by the Tukey test

BMI body mass index, *CA* chronological age, *BA* bone age

the three biomarkers were higher in the 13 to 15-year age group, a period corresponding to the PHV in boys.

Analysis of the three biomarkers according to nutritional status, sex, and age group showed no significant differences in the biomarkers between overweight, obese and extremely obese adolescents.

When the same analysis was performed in adolescent boys (Table 4), significant differences were only observed in mean S-CTx levels for the age groups between 10–12 and 13–15 years, with higher levels between overweight and obese adolescents in the 10 to 12-year group and between obese and extremely obese adolescents in the 13 to 15-year group.

Table 5 compares the bone biomarkers (BAP, osteocalcin, and S-CTx) between male and female adolescents classified based on BMI as normal weight and excess weight according to BA group. In girls, significant differences in BAP were observed between normal weight adolescents and adolescents with excess weight in the 10 to 11-year (BA1), 12 to 13-year (BA2) and 16 to 19-year (BA4) groups, but not in the 14 to 15-year (BA3) group. Regarding osteocalcin, differences between normal weight and excess weight adolescents were observed in the 14 to 15-year (BA3) and 16 to 19-year (BA4) groups. Finally, analysis of the profile of S-CTx showed differences between normal weight and excess weight adolescents only in the older age group (16 to 19 years, BA4).

Table 2 Characterization of male and female adolescents according to weight group (mean and standard deviation) adjusted for chronological age and bone age

Weight groups—female (*n* = 208)						
Variables	Normal weight (*n* = 72)	Overweight (*n* = 53)	Obese (*n* = 62)	Extremely obese (*n* = 21)	P (CA)	P (BA)
Weight (kg)	47.74 ± 9.57^{aA}	59.59 ± 9.61^{bB}	71.28 ± 13.19^{cC}	88.89 ± 12.09^{dD}	0.0001	0.0001
Height (m)	1.56 ± 0.10^{aA}	1.58 ± 0.08^{aA}	1.58 ± 0.07^{aA}	1.57 ± 0.05^{aA}	0.4915	0.5275
BMI (kg/m^2)*	19.35 ± 2.45^{aA}	23.72 ± 1.71^{bB}	28.45 ± 3.79^{cC}	36.00 ± 6.02^{dD}	0.0001	0.0001
Lean mass (g)	31820.48 ± 5715.62^{bB}	37974.13 ± 5482.44^{aA}	43649.20 ± 7428.82^{dAC}	49140.79 ± 4824.06^{dAC}	0.0001	0.0001
Fat mass (g)	13732.60 ± 4864.02^{aA}	19962.32 4824.5^{bB}	26351.65 ± 6613.80^{cC}	35386.46 6811.09^{dD}	0.0001	0.0001
BF (%)**	28.49 ± 5.07^{aA}	32.98 ± 4.31^{bB}	36.65 ± 4.06^{cC}	40.86 ± 4.11^{dD}	0.0001	0.0001
Weight groups—male (*n* = 183)						
Variables	Normal weight (*n* = 86)	Overweight (*n* = 29)	Obese (*n* = 43)	Extremely obese (*n* = 25)	P (CA)	P (BA)
Weight (kg)	50.62 ± 12.22^{aA}	62.91 ± 13.26^{bB}	75.53 ± 13.25^{cC}	87.04 ± 7.95^{dD}	0.0001	0.0001
Height (m)	1.63 ± 0.12^{aA}	1.64 ± 0.12^{aA}	1.64 ± 0.10^{aA}	1.63 ± 0.06^{aA}	0.9761	0.0788
BMI (kg/m^2)*	18.73 ± 2.55^{aA}	23.11 ± 1.72^{bB}	27.76 ± 2.78^{cC}	33.48 ± 2.73^{dD}	0.0001	0.0001
Lean mass (g)	38161.72 ± 10413.12^{bB}	45614.87 ± 11804.59^{aA}	50284.30 ± 10102.04^{acA}	52679.58 ± 657.62^{cA}	0.0001	0.0001
Fat mass (g)	8535.58 ± 2668.21^{bB}	16211.66 ± 4590.15^{dD}	23778.60 4915.22^{cC}	31822.86 ± 6459.29^{aA}	0.0001	0.0001
BF (%)**	18.56 ± 4.77^{aB}	26.41 ± 6.61^{bC}	31.55 ± 5.18^{cA}	36.18 ± 6.05^{dA}	0.0001	0.0001

Different letters indicate significant differences among groups (normal weight, overweight, obese and extremely obese). Same letters indicate no significant differences among groups (normal weight, overweight, obese and extremely obese). Lowercase letters: comparison by CA. Uppercase letters: comparison by BA. ANOVA followed by the Tukey test

BMI body mass index, *BF* body fat percentage, *CA* chronological age, *BA* bone age

Table 3 Bone biomarkers (BAP, OC, S-CTx) of male and female adolescents with excess weight ($n = 233$) according to bone age

Bone biomarkers										
Age	*n*	Mean BAP (U/L)	SD	Median	Mean OC (ng/mL)	SD	Median	Mean S-CTx (ng/mL)	SD	Median
Female ($n = 136$)										
10–11	10	16.46	33.64	165.85	37.85	11.61	35.88	1.56	0.41	1.55
12–13	38	163.16	55.42	161.50	31.88	16.61	31.65	1.51	0.45	1.48
14–15	28	75.63	40.29	58.51	22.51	12.68	20.79	0.97	0.37	0.93
16–19	60	58.73	50.64	47.38	16.40	9.21	14.05	0.71	0.26	0.66
Male ($n = 97$)										
10–12	8	166.53	52.22	172.62	26.67	10.73	25.99	1.78	0.49	1.86
13–15	64	186.10	73.24	190.52	34.27	15.40	30.93	1.77	0.49	1.77
16–19	25	88.85	43.19	84.50	23.28	11.99	19.99	1.08	0.36	1.09

Age groups according to bone age

BAP bone alkaline phosphatase, *OC* osteocalcin, *S-CTx* carboxy-terminal telopeptide

When the same analysis was performed in male adolescents, significant differences in BAP between normal weight and excess weight adolescents were only observed in the 13 to 15-year (BA2) group. In contrast, differences in osteocalcin between normal weight and excess weight adolescents occurred in the 16 to 19-year (BA3) group. Analysis of the profile of S-CTx revealed differences between normal weight and excess weight adolescents in the 13 to 15 -year (BA2) and 16 to 19-year (BA3) groups.

Table 6 shows the Pearson correlations of bone biomarkers with the nutritional variables and densitometry parameters for the final sample of adolescents of both sexes with excess weight. In girls, significant negative correlations were observed between LM, FM and BF% and each of the three bone biomarkers, i.e., the higher BF% and FM, the lower the concentrations of BAP, osteocalcin and S-CTx. Furthermore, BMI and lumbar spine, femoral, subtotal body and total body BMC and BMD were negatively and significantly correlated with each of the three bone biomarkers.

In contrast, no correlations between LM or FM and the three biomarkers were observed in boys, except for BF%, which was significantly correlated with osteocalcin. However, there was a significant negative correlation of BMC (lumbar spine, subtotal body, and total body) and BMD (lumbar spine and total body) with BAP and S-CTx. Osteocalcin only showed a significant negative correlation with lumbar spine BMC.

Discussion

The main findings of this study were that the static evaluation of bone mass, represented by BMC and BMD at specific sites and of the whole body, was inversely correlated with almost all bone biomarkers in adolescent boys and girls with excess weight. Furthermore, the results showed that excess body fat (BF% and FM) had a negative impact on the profile of biomarkers in female adolescents (Table 6). Additionally, the bone biomarkers were found to follow the age groups for growth velocity, in which bone formation and resorption were increased in the first years of puberty in boys and girls and decreased significantly with increasing age (Table 3). Despite the cross-sectional design of the present study, the sample size was considerable ($n = 391$), the study included a nutritional risk group, and static and dynamic evaluations of bone status were performed. Moreover, childhood and adolescence are the exclusive periods of longitudinal physical growth characterized by intense bone modeling and remodeling [23]. Thus, our results permit a better understanding of the mechanisms of bone turnover in a group with a serious nutritional problem.

In an attempt to clarify the possible effects of excess body fat on variables related to bone formation and resorption, we observed significant negative correlations between FM (g) and BF% and the three bone biomarkers, especially osteocalcin, in girls. The results confirmed data in the literature on this bone marker [24] obtained for Korean children, showing that the higher the excess weight, the lower the level of osteocalcin. The significant and negative correlations between FM and the three bone biomarkers found in the present study in girls may reflect the complex interaction between bone biomarkers, bone mass and gonadal steroids during puberty [25]. These authors observed differences in the association between bone mass and osteocalcin when the pre-and mid-pubertal periods were compared, with the observation of an inverse relationship only in mid-puberty. Another interesting result was related to the strength of the correlation between osteocalcin and total body bone mass, with an r value of −0.60 in eutrophic girls, while eutrophic boys had a correlation of −0.07 [25]. These results indicate differences in the magnitude of the impact of bone mass between sexes.

Table 4 Mean, standard deviation and median of the bone biomarkers (BAP, OC, S-CTx) in female and male adolescents with excess weight according to weight group (body mass index) and age group

Age	n	mean	SD	median	n	mean	SD	median	n	Mean	SD	median	Total	P
Female ($n = 208$)														
	Overweight ($n = 53$)				Obese ($n = 62$)				Extremely obese ($n = 21$)					
BAP (U/L)														
10–11	4	167.67	33.83	165.86	5	161.71	40.44	167.31	1	150.59	–	150.58	10	0.895
12–13	18	162.50	62.59	159.57	14	158.30	46.29	162.72	6	176.48	59.63	161.87	38	0.841
14–15	8	80.84	41.26	74.69	15	68.29	40.61	53.97	5	89.03	43.22	72.30	28	0.571
16–19	23	52.63	32.62	41.77	28	65.37	64.94	51.60	9	49.65	18.19	41.56	60	0.271
OC (ng/mL)														
10–11	4	43.28	13.78	35.98	5	35.74	11.77	36.04	1	32.11	–	32.11	10	0.551
12–13	18	32.87	19.44	29.85	14	31.41	12.57	31.17	6	29.98	18.37	34.53	38	0.946
14–15	8	28.83	12.30	26.54	15	19.46	14.16	17.38	5	20.32	4.74	20.24	28	0.346
16–19	23	17.67	10.85	13.52	28	16.72	8.66	14.98	9	13.05	7.49	9.92	60	0.374
S-CTx (ng/mL)														
10–11	4	1.48	0.61	1.28	5	1.61	0.38	1.63	1	1.63	–	1.63	10	0.897
12–13	18	1.60	0.46	1.52	14	1.39	0.40	1.48	6	1.50	0.53	1.50	38	0.462
14–15	8	0.98	0.29	0.89	15	0.90	0.32	0.93	5	1.16	0.64	1.06	28	0.443
16–19	23	0.73	0.30	0.72	28	0.72	0.25	0.66	9	0.62	0.20	0.57	60	0.457
Male ($n = 183$)														
	Overweight ($n = 29$)				Obese ($n = 43$)				Extremely obese ($n = 25$)					
BAP (U/L)														
10–12	3	199.72	32.60	199.72	3	145.65	23.40	133.50	2	164.64	103.39	164.65	8	0.471
13–15	15	201.26	105.27	146.19	30	182.66	65.91	195.38	19	181.59	61.67	178.36	64	0.776
16–19	11	96.37	53.78	82.48	10	74.76	27.06	78.33	4	105.49	51.15	127.55	25	0.366
OC (ng/mL)														
10–12	3	30.68	6.62	30.68	3	24.42	15.58	20.01	2	26.03	10.65	26.04	8	0.807
13–15	15	29.66	17.58	28.01	30	34.67	14.58	32.06	19	36.60	15.36	31.07	64	0.502
16–19	11	23.10	11.92	23.14	10	19.27	6.44	16.68	4	32.67	18.45	36.13	25	0.164
S-CTx (ng/mL)														
10–12	3	2.19^{A}	0.44	2.19	3	1.92^{A}	0.13	1.92	2	1.24^{B}	0.24	1.24	8	0.008
13–15	15	1.40^{B}	0.44	1.30	30	1.84^{A}	0.46	1.81	19	1.89^{A}	0.47	1.78	64	0.008
16–19	11	1.08	0.36	1.10	10	1.00	0.25	0.97	4	1.24	0.60	1.36	25	0.554

Multiple comparison using PROC GENMOD of the SAS program. Different letters indicate significant differences among groups (overweight, obese and extremely obese) at 5%, Same letters indicate no significant differences among groups (overweight, obese and extremely obese) at 5%. Age groups according to bone age

BAP bone alkaline phosphatase, *OC* osteocalcin, *S-CTx* carboxy-terminal telopeptide

In addition, Dubnov-Raz et al. [26] questioned whether the relationship between osteocalcin and BMI is dependent on bone tissue or is related to fat tissue, since the latter is also able to produce and secrete osteocalcin [26]. Clearly, more studies are needed to elucidate the mechanisms underlying this difference, especially studies including obese and extremely obese adolescents. This aspect has been recently addressed by a case-control study that demonstrated suppressed bone turnover in obese adults with severe childhood-onset obesity [27].

Within this context, we can highlight a recent longitudinal study investigating associations between biochemical markers of bone and adipose tissue during puberty [28]. The sample consisted of 96 boys evaluated at three time points: baseline and 12 and 24 months of follow-up. The results showed that the bone metabolism markers had a negative effect on bone mineral accrual during puberty and that increases in physical activity level affected leptin, suggesting a positive effect of physical training through leptin metabolism on increases in bone mineralization during puberty in boys [28]. Despite its cross-sectional design, using a homogenous sample in terms of habitual physical activity level in which all nutritional groups were classified as sedentary, the present study demonstrated the impact of adiposity on bone mass and bone biomarkers.

Table 5 Bone biomarkers (BAP, OC, S-CTx) of normal weight and excess weight female and male adolescents according to age group

Age group	*n*	Mean	SD	*n*	Mean	SD	*P*
Female (*n* = 208)							
	Normal weight (*n* = 72)			Excess weight (*n* = 136)			
BAP (U/L)							
BA_1	12	108.59^B	31.41	10	162.46^A	33.64	0.039
BA_2	16	114.91^B	42.15	38	163.16^A	55.42	0.017
BA_3	15	66.54^A	33.13	28	75.63^A	40.29	0.383
BA_4	29	34.17^B	13.62	60	58.73^A	50.64	0.001
OC (ng/mL)							
BA_1	12	47.69	26.66	10	37.85	11.61	0.381
BA_2	16	23.19	19.55	38	31.88	16.61	0.199
BA_3	15	8.98^B	6.35	28	22.51^A	12.68	0.001
BA_4	29	5.85^B	3.47	60	16.40^A	9.21	0.001
S-CTx (ng/mL)							
BA_1	12	1.85	0.58	10	1.56	0.41	0.292
BA_2	16	1.38	0.78	38	1.51	0.45	0.416
BA_3	15	0.77	0.23	28	0.97	0.37	0.052
BA_4	29	0.52^B	0.20	60	0.71^A	0.26	0.001
Male (*n* = 183)							
	Normal weight (*n* = 86)			Excess weight (*n* = 97)			
BAP (U/L)							
BA_1	16	111.42	42.41	8	166.53	52.22	0.054
BA_2	44	151.47^B	71.10	64	186.10^A	73.24	0.036
BA_3	26	89.85	49.67	25	88.85	43.19	0.937
OC (ng/mL)							
BA_1	16	30.02	9.15	8	26.67	10.73	0.579
BA_2	44	37.86	15.20	64	34.27	15.40	0.275
BA_3	26	31.69^A	16.69	25	23.28^B	11.93	0.027
S-CTx (ng/mL)							
BA_1	16	1.68	0.24	8	1.78	0.490.671	
BA_2	44	2.01^A	0.57	64	1.77^B	0.490.044	
BA_3	26	1.87^A	0.87	25	1.08^B	0.36	0.001

BA_1 = 10–11 years; BA_2 = 12–13 years; BA_3 = 14–15; BA_4 = 16–19 years. Different letters indicate significant differences among groups (normal weight and overweight). Same letters indicate no significant differences among groups (normal weight and overweight). Age groups according to bone age (BA). Normal weight: $BA_1 = BA_2 > BA_3 > BA_4$. Overweight: $BA_1 = BA_2 > BA_3 > BA_4$

BAP bone alkaline phosphatase, *OC* osteocalcin, *S-CTx* carboxy-terminal telopeptide

In the present study, regarding sex, we found no significant differences in the biomarkers of bone remodeling between overweight, obese, and extremely obese female adolescents in the different age groups proposed. On the other hand, in boys, differences were observed for S-CTx in the early age groups (10 to 12 years), with higher mean values indicating greater bone resorption in overweight and obese adolescents, and in the 13 to 15-year age group, with higher values in obese and extremely obese adolescents. The BAP concentrations were significantly higher in girls with excess weight compared to their normal weight counterparts in almost all age groups. The same was observed for osteocalcin and S-CTx, but only in the more advanced age groups. This increase in S-CTx can again be explained by the chronic inflammatory response caused by excess weight, in which inflammatory cytokines produced by adipose tissue, including IL-1α, IL-1β, IL-6, and TNF-α, induce a process called osteoclastogenesis. These cytokines are notoriously elevated in obese individuals, causing greater bone resorption and consequently decreasing bone mass accrual in these obese adolescents during puberty [29, 30]. In contrast, in boys, BAP concentrations were significantly higher in adolescents with excess weight only in the 13 to 15-year age group. On the other hand, osteocalcin and S-CTx levels were significantly lower in boys with excess weight compared to normal weight adolescents, but in the more advanced age groups. The latter fact suggests lower bone formation in these individuals. The same trend was reported by Viljakainen et al. [27]. In general, our findings agree with those reported in the cross-sectional study of Dimitri et al. [9] in which negative associations between bone biomarkers and excess body weight in adolescents are the result of multiple factors. In particular, adipose tissue releases some adipokines (cytokines) that have been shown to play an important role in growth regulation within the context of obesity. Among these adipokines, leptin is particularly interesting because it is involved in the onset and progression of puberty and is highly correlated with the amount of body fat. Furthermore, an association between obesity and impaired GH secretion in girls has been suggested by De Leonibus et al. [31]. In contrast, in boys with adequate weight for age, serum leptin declines after the onset of puberty due to higher lean mass acquisition, returning to normal levels about 30 to 40 months after this phase [29].

It is also known that elevated leptin concentrations can have a negative impact on bone metabolism in obese children and adolescents, since elevated concentrations accelerate the process of bone resorption and reduce bone formation. The consequences are alterations in the bone microstructure of these individuals and an increased fracture risk [32]. However, these results are still contradictory and inconclusive since the serum concentrations of adipokines can be affected by multiple factors such as age, sex, race, smoking, the presence of diseases (diabetes), hormone levels, and physical activity. In this respect, despite adjustment for age, sex, and other variables, all studies have limitations that could influence the results obtained regarding alterations in microarchitecture and the observation of bone fractures [27]. In fact, it is recognized that a complex interaction exists between bone and adipose tissue that is potentiated during a certain period of puberty.

Table 6 Pearson correlation between bone biomarkers (BAP, OC, S-CTx) and body mass index, lean mass, fat mass, body fat percentage, bone mineral content, and bone mineral density evaluated at four sites in the final sample of female and male adolescents with excess weight

	Female ($n = 136$)			Male ($n = 97$)		
	BAP (U/L)	OC (ng/mL)	S-CTx (ng/mL)	BAP (U/L)	OC (ng/mL)	S-CTx (ng/mL)
BMI (kg/m^2)	−0.392 (<0.001)	−0.398 (<0.001	−0.441 (<0.001)	−0.172 (0.085)	−0.001 (0.999)	−0.055 (0.590)
LM (g)	−0.498 (<0.001)	−0.446 (<0.001)	−0.497 (<0.001)	−0.200 (0.050)	−0.181 (0.078)	−0.197 (0.058)
FM (g)	−0.461 (<0.001)	−0.449 (<0.001)	−0.431 (<0.001)	−0.001 (0.996)	0.132 (0.201)	0.045 (0.668)
BF (%)	−0.204 (0.016)	−0.259 (0.002)	−0.189 (0.028)	0.072 (0.478)	0.216 (0.031)	0.135 (0.190)
BMC-Spine (g/cm^2)	−0.587 (<0.001)	−0.467 (<0.001)	−0.554 (<0.001)	−0.358 (0.001)	−0.216 (0.031)	−0.321 (0.001)
BMC-Femur (g/cm^2)	−0.452 (<0.001)	−0.352 (<0.001)	−0.445 (<0.001)	−0.137 (0.175)	−0.114 (0.259)	−0.110 (0.282)
BMC-Subtotal (g/cm^2)	−0.565 (<0.001)	−0.452 (<0.001)	−0.547 (<0.001)	−0.299 (0.003)	−0.115 (0.258)	−0.278 (0.006)
BMC-Total (g/cm^2)	−0.596 (<0.001)	−0.462 (<0.001)	−0.573 (<0.001)	−0.243 (0.016)	−0.061 (0.551)	−0.253 (0.013)
BMD-Spine (g/cm^2)	−0.59645 (<0.001)	−0.458 (<0.001)	−0.553 (<0.001)	−0.390 (<0.001)	−0.117 (0.247)	−0.360 (0.003)
BMD-Femur (g/cm^2)	−0.505 (<0.001)	−0.396 (<0.001)	−0.487 (<0.001)	−0.178 (0.077)	−0.123 (0.222)	−0.246 (0.015)
BMD-Subtotal (g/cm^2)	−0.581 (<0.001)	−0.431 (<0.001)	−0.540 (<0.001)	−0.342 (0.006)	−0.137 (0.178)	−0.383 (0.001)
BMD-Total (g/cm^2)	−0.629 (<0.001)	−0.432 (<0.001)	−0.581 (<0.001)	−0.387 (<0.001)	−0.128 (0.207)	−0.459 (<0.001)

Pearson correlation test

BAP bone alkaline phosphatase, *OC* osteocalcin, *S-CTx* carboxy-terminal telopeptide, *BMI* body mass index, *LM* lean mass, *FM* fat mass, *BF* (%) body fat percentage, *BMC* bone mineral content, *BMD* bone mineral density

Longitudinal studies with follow-up in puberty are necessary to identify the mechanisms that remain unclear.

Finally, the biomarker trends demonstrated across the age groups of the present study follow the age trends for growth velocity. These trends may be considered to occur in parallel with the acceleration and deceleration of growth which is typical in this phase of life, as reported by Bayer [33]. This author determined reference values of plasma osteocalcin and procollagen type I N-propeptide in 439 Caucasian individuals, including 232 boys and 207 girls (age range 0–18 years). The results showed that bone metabolism activity is very high during the first year and then declines until 3 years of age, remaining relatively stable until the pubertal growth spurt. Biomarkers increased during early puberty and reached maximal levels at stages 2 and 3 in boys and girls [33]. This trend was observed in the present study in adolescents with excess weight and has been reported in other studies evaluating normal weight adolescents of both sexes [16, 34–36].

We found higher BAP, osteocalcin, and S-CTx levels in the age groups that contained the PHV in the total sample of adolescents with excess weight. In girls, this period comprises the ages of 10 to 13 years, which correspond to the years of maximum acceleration of growth. However, in the case of Brazilian boys, studies have shown that the PHV occurs at around 13.3 years, an age included in the second age group, 13 to 15 years, in which the mean levels of the three biomarkers were also higher when compared to the other age groups. This may indicate an important association between PHV and peak bone mineral content velocity, in agreement with other authors [37]. With respect to bone mass accrual, Vatanparast and Whiting [38] found that peak bone mineral content velocity occurs on average 0.7 years after PHV and is clearly related to the maturation of adolescents. Additionally, we highlight that mean BA advancement over mean CA was observed in boys and girls of all weight groups. This observation has also been pointed out for girls in the specialized literature [28], but controversies exist for boys [39]. Recently, Vandewalle et al. [40] confirmed these findings, which were also observed in the present study.

Within this context, it is recognized that the interpretation of markers of bone turnover in adolescents is not an easy task as they simultaneously reflect growth, remodeling, and nutritional status. Thus, as a limitation, we emphasize that cross-sectional studies only provide hypotheses and associations and prospective cohort studies are necessary to identify causal relationships. In addition, the results of dietary recalls on the intake of calcium and vitamin D in this population, although obtained, are not shown. However, previously published studies have shown deficiency of these nutrients in both normal weight and obese individuals [8, 41, 42]. Furthermore, interpretation of the evolution of bone markers permitted to observe the events of bone formation and resorption through the measurement of these markers. However, unfortunately, the results do not permit us to distinguish their activity between the phases of bone growth, modeling, and remodeling. Furthermore, the markers only represent the mean of all these events that occur simultaneously during this period of life, with different rates in the various skeletal sites [43].

In view of these observations and inferences, we suggest that these are mechanisms of protection developed by the

organism to cope with this metabolically serious situation, characterized by a high prevalence and different degrees of obesity in individuals who are still in the phase of growth and physical development, in an attempt to preserve bone mass now and later in life.

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Compliance with ethical standards

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Conflicts of interest None.

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