

## Relationship between bone mineral density, leptin and insulin concentration in Brazilian obese adolescents

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**Abstract** Despite the epidemic of adolescent obesity, the effect of obesity and hormones on bone mineral accrual during growth is poorly understood. Studies using dual-energy X-ray to examine the effect of obesity on bone mass in children and adolescents have yielded conflicting results. The aim of this study was to explore the combined and independent contributions of body mass index, body composition, leptin, insulin, glucose levels and Homeostasis Model Assessment Insulin Resistance (HOMA-IR) to bone mineral density (BMD) and bone mineral content in a group of Brazilian obese adolescents. This study included 109 post-pubescent obese adolescents. A whole-body dual-energy X-ray absorptiometry scan was performed, using a HOLOGIC QDR4200, to determine whole-body BMD and

body composition. Blood samples were collected in the outpatient clinic after an overnight fast, and evaluated for fasting blood glucose and immunoreactive insulin. Leptin levels were assessed with a radioimmunoassay kit. Insulin resistance was assessed by HOMA-IR and the quantitative insulin sensitivity check index. Our results showed that insulin levels and HOMA-IR correlated negatively with BMD and a linear regression analysis showed that serum leptin is inversely associated to BMD adjusted for body mass. In conclusion, our data support the hypothesis that leptin, insulin and HOMA-IR are inversely associated with BMD and play a significant direct role in bone metabolism.

**Keywords** Obesity · Bone mass · Hormones · DEXA · Body composition

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### Introduction

Bone remodeling is a complex process characterized by an ongoing coordinated reabsorption and formation of new bone, regulated by systemic hormones and local factors. The fact that bone remodeling occurs independently at multiple skeletal locations has generally been viewed as indirect evidence for local regulation [1]. As a result of mounting evidence suggesting beneficial effects on bone, increased body weight has emerged in recent years as a potential modifier of osteoporosis risk [2, 3]. Likewise, body mass index (BMI), has been accorded the same attention [4].

Bone-protective effects of obesity may involve increased weight bearing, increased aromatization of androgen to estrogen in adipose tissue, lowered levels of sex hormone binding globulin (SHBG), or a direct

increased bone formation induced by high circulating levels of insulin [1].

Krakauer et al. [5] concluded that the metabolic effects of poor glycemic control lead to increased bone resorption and bone loss in young diabetic adults. Improvements in glycemic control increased values of serum osteocalcin in diabetic patients. These studies suggest that during periods of poor glycemic control, increased rates of bone resorption are not accompanied by proportional increases in rates of bone formation [6].

In addition, a growing body of work has established the central role of the hypothalamus in the regulation of bone formation by osteoblasts [7]. The thrust of this influence is exerted by neurons located in the ventromedial hypothalamus. These neurons are themselves the target of leptin, which was demonstrated to be a powerful antiosteogenic hormone [7]. Based on chemical lesioning, genetic manipulations, and pharmacological experiments, hypothalamic neurons mediating leptin antiosteogenic and anorexigenic functions could be distinguished. Moreover, genetic evidence indicates that, peripherally, the sympathetic nervous system preferentially mediates leptin antiosteogenic function [8].

Leptin, the product of the *Ob* gene, is primarily released by white adipose tissue and is strongly correlated with fat mass [9]. Leptin receptors have been discovered in human bone marrow stromal cells and are known to induce the differentiation of stromal cells to osteoblasts [10]; however, human studies on the role of leptin in bone metabolism have shown contradictory results [1, 11, 12]. A recent study has suggested that the effect of leptin signaling on bone might differ significantly between different skeletal regions [13], and this might partially explain the contradictory results of research on the association of leptin and bone metabolism.

During childhood and adolescence, bone mineral accretion results in sex- and maturation-specific increases in cortical dimensions and trabecular density [14]. These periods are crucial for bone growth, considering half the adult bone mass is achieved in adolescence. Indeed, the peak bone mass of early adulthood may provide an important protection against osteoporotic fracture risk occurring later in life, reinforcing the importance of detecting children with low bone mass to schedule intervention programs at an early age [15]. Despite the epidemic of adolescent obesity, the effect of obesity and hormones on bone mineral accrual during growth is poorly understood. Studies using dual-energy X-ray (DEXA) to examine the effect of obesity on bone mass in children and adolescents have yielded conflicting results.

In adults, obesity is associated with increased bone mineral density (BMD), and with decreased risk of hip fracture in postmenopausal women [16]. Although the

association of obesity with bone mineral content (BMC) and BMD in children and adolescents is unclear and controversial: some studies revealed higher incident of fracture risk in obese children [17–19]. Thus, the aim of this study was to explore the combined and independent contributions of BMI, body composition, leptin, insulin, glucose levels and HOMA-IR to BMD and BMC in Brazilian obese adolescents.

## Research methods

For this study, 109 adolescents (41 males and 68 females) with simple obesity were recruited. Their ages ranged from 13 to 18 years. The inclusion criteria were Tanner Stage 5 assessed by means of the Tanner criteria [20] and primary obesity, BMI greater than 30 wt/ht<sup>2</sup>. Exclusion criteria included current or previous (within 6 months) use of oral contraceptive pills, cortisone, anti-epileptic drugs, cholesterol-lowering drugs or binders, history of renal, gastrointestinal, or liver disease, alcohol intake, ethanol more than two drinks per day, smoking, current or anticipated pregnancy, obesity secondary to endocrine disease, participation in any strenuous physical exercise, chronic diseases, history of fractures or long-term immobilization, and supplementation of calcium or any other drug that might affect their bone metabolism.

Standing height and weight were measured and BMI was calculated as body weight (wt) divided by height (ht) squared (wt/ht<sup>2</sup>). The mean BMI was  $35.64 \pm 4.35$  wt/ht<sup>2</sup>. Blood samples were collected in the outpatient clinic around 8:00 A.M. after an overnight fast, and fasting blood glucose (FBG) immunoreactive insulin (IRI) were recorded. Leptin levels were assessed with an RIA kit (Linco Research, St. Charles, Missouri, United States) [21]. Insulin resistance was assessed by homeostasis model assessment insulin resistance index (HOMA-IR) [22] and the quantitative insulin sensitivity check index (QUICKI) [23]. HOMA-IR was calculated as [FBG (mg/dl) X IRI (mU/l)]/405. All parameters were analyzed according to reference values described by Schwimmer et al. [24]. This study was carried out in accordance with the principles of the Declaration of Helsinki and was approved by an ethical committee under number #0135/04. Informed consent was obtained from all subjects and/or their parents.

## Bone mineral density and body composition

A whole-body dual-energy X-ray absorptiometry scan was performed to determine whole-body BMD (units: g/cm<sup>2</sup>) and body composition using HOLOGIC QDR4200 (Hologic, Bedford, MA). The whole-body scan requires the

subject to be placed supine with the arms and legs positioned according to the manufacturer's specifications. Quality control was performed daily using a phantom, and measurements were maintained within the manufactures standards of  $\leq 1\%$  [25].

### Statistical analyses

Data are presented as means  $\pm$  standard deviation. *P* values less than .05 are considered as statistically significant. Descriptive analysis and evaluation of normality were performed first. Correlation analyses between body composition, BMI, fasting glucose, insulin, leptin, HOMA-IR with BMD and BMC were performed with Pearson's correlation test. Stepwise multiple regression of BMD and BMC as dependent factors were performed. All analyses were realized in STATISTICAL 6 for Windows.

### Results

The characteristics of all obese adolescents are show in Table 1. As expected, boys were taller, heavier, with higher values of BMC and more free fat mass than girls. On the other hand girls presented more fat mass percentage and leptin values; for the other variables no differences were found.

No correlations were found, in either sex, between BMD and BMC with body mass, fat mass (kg), free fat mass (%), subcutaneous fat, glucose and leptin. However, for boys, strongly positive correlations were observed between BMD and height ( $r = 0.724$ ) and absolute free fat mass ( $r = 0.798$ ); and negative correlations with visceral fat ( $r = -0.658$ ), insulin ( $r = -0.730$ ) and HOMA-IR ( $r = -0.705$ ). Indeed, we verified a tendency for negative correlation between BMD and fat mass percentage ( $P = 0.05$ ;  $r = -0.531$ ) in the male subjects. In girls, BMD presented positive correlations with age ( $r = 0.479$ ) and absolute free fat mass ( $r = 0.365$ ). No negative correlations were observed for girls (Table 2).

Linear regression analysis showed, only in boys, that serum leptin is inversely associated to BMD adjusted for body mass ( $r = -0.53$ ;  $P \leq 0.039$ ) (Fig. 1).

In Table 2, we can observe the correlation with BMC and all variables. For boys there was a positive correlation between BMC and height ( $r = 0.731$ ) and a strong correlation with absolute free fat mass ( $r = 0.815$ ); and negative correlation between visceral fat ( $r = -0.613$ ), insulin ( $r = -0.747$ ) and HOMA-IR ( $r = -0.724$ ). For girls positive correlations were observed for age ( $r = 0.467$ ) and absolute free fat mass ( $r = 0.618$ ). Once again, no negative correlations were found in juvenile females.

**Table 1** Anthropometric, body composition and biochemical parameters in 109 obese adolescents

	Boys ( <i>n</i> = 41)	Girls ( <i>n</i> = 68)	<i>P</i> value
Age (years)	17.07 $\pm$ 1.61	16.70 $\pm$ 1.67	NS
Body mass (kg)	106.86 $\pm$ 12.05	92.76 $\pm$ 12.47	0.000000
Height (m)	1.72 $\pm$ 0.07	1.62 $\pm$ 0.05	0.000000
BMI (kg/m <sup>2</sup> )	36.03 $\pm$ 3.75	35.09 $\pm$ 4.06	NS
Fat mass (%)	37.01 $\pm$ 7.32	44.71 $\pm$ 5.14	0.000000
Fat mass (kg)	39.36 $\pm$ 10.35	40.74 $\pm$ 8.83	NS
Free fat mass (%)	62.48 $\pm$ 8.16	55.28 $\pm$ 5.13	0.000001
Free fat mass (kg)	65.80 $\pm$ 8.49	49.60 $\pm$ 5.22	0.000000
Visceral fat (cm)	4.42 $\pm$ 1.36	3.54 $\pm$ 1.30	0.001
Subcutaneous fat (cm)	2.99 $\pm$ 0.66	3.28 $\pm$ 0.86	NS
BMD	1.17 $\pm$ 0.14	1.14 $\pm$ 0.08	NS
BMC	2657.79 $\pm$ 523	2384.82 $\pm$ 307	0.007
Glucose (mg/dl)	92.00 $\pm$ 6.50	91.39 $\pm$ 6.43	NS
Insulin (uU/ml)	19.06 $\pm$ 13.19	17.32 $\pm$ 8.14	NS
HOMA-IR	4.42 $\pm$ 3.34	3.90 $\pm$ 1.97	NS
Leptin (ng/dl)	18.66 $\pm$ 9.06	36.34 $\pm$ 17.39	0.000001

Reference values: leptin (4.9–24 ng/dl) [21]; glucose (70–100 mg/dl) [24]; insulin (<17 uU/ml) [25]; HOMAR-IR (<3) [25]

*BMI* Body mass index, *BMD* Bone mineral density, *BMC* Bone mineral content, *HOMA-IR* Homeostasis model assessment insulin resistance index

According to Table 3 there was a positive correlation, only in girls, between FFM and insulin concentration ( $r = 0.28$ ;  $P \leq 0.036$ ).

Stepwise multiple linear regression analyses were performed. Tables 4 and 5 (boys and girls respectively) present multiple regression analysis with BMD as the dependent variable. The absolute free fat mass and visceral fat provided the best model to explain variability in bone density in boys; in girls the best predictor was the absolute free fat mass.

The same multiple linear regression analyses were performed using BMC as the dependent variable, and models are within 2 steps for boys and 3 for girls. In Table 5, we can observe that absolute free fat mass and insulin, in boys, and absolute free fat mass and visceral fat in girls, were the best values for determining BMC.

### Discussion

A number of different factors are listed as being important for growth of BMD during puberty. Among these factors, those that stand out are contributions of natural genetics, alterations to the body's dimensions, weight and stature, physical activity level, hormonal profile and sufficient calcium ingestion during this age range; all these are reflected in intense bone mineralization [26, 27].

**Table 2** Simple correlation coefficient between bone mineral density and bone mineral content with anthropometrics measurements, body composition and biochemical parameters in obese adolescents

	Boys ( <i>n</i> = 41)		Girls ( <i>n</i> = 68)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
<b>Bone mineral density</b>				
Age (years)	0.402	0.153	0.479	0.006
Body mass (kg)	−0.098	0.737	0.215	0.237
Height (m)	0.724	0.003	0.028	0.879
BMI (kg/m <sup>2</sup> )	−0.527	0.053	0.238	0.189
Fat mass (%)	−0.531	0.050	−0.031	0.865
Fat mass (kg)	−0.392	0.165	0.146	0.425
Free fat mass (%)	0.387	0.171	0.031	0.865
Free fat mass (kg)	0.798	0.001	0.365	0.040
Visceral fat (cm)	−0.658	0.010	−0.314	0.080
Subcutaneous fat (cm)	−0.167	0.568	0.077	0.672
Glucose (mg/dl)	−0.327	0.253	0.195	0.284
Insulin (uU/ml)	−0.730	0.003	−0.066	0.718
HOMA-IR	−0.705	0.005	−0.036	0.843
Leptin (ng/dl)	−0.310	0.280	0.040	0.827
<b>Bone mineral content</b>				
Age (year)	0.369	0.194	0.467	0.007
Body mass (kg)	−0.052	0.858	0.379	0.032
Height (m)	0.731	0.003	0.312	0.082
BMI (kg/m <sup>2</sup> )	−0.504	0.066	0.279	0.122
Fat mass (%)	−0.492	0.074	−0.147	0.419
Fat mass (kg)	−0.356	0.211	0.143	0.434
Free fat mass (%)	0.341	0.233	0.147	0.419
Free fat mass (kg)	0.815	0.000	0.618	0.000
Visceral fat (cm)	−0.613	0.020	−0.324	0.070
Subcutaneous fat (cm)	−0.237	0.414	0.102	0.577
Glucose (mg/dl)	−0.341	0.233	0.186	0.306
Insulin (uU/ml)	−0.747	0.002	0.070	0.702
HOMA-IR	−0.724	0.003	0.098	0.593
Leptin (ng/dl)	−0.343	0.230	−0.024	0.893

*BMI* Body mass index, *BMD* Bone mineral density, *BMC* Bone mineral content, *HOMA-IR* Homeostasis model assessment insulin resistance index

With respect to anthropometric variables important to bone mineralization in adolescence, there are conflicting results regarding the influence of body height, body mass and BMI on BMD in different populations studied. Jüremäe et al. [28] verified associations between anthropometric variables and BMD, but effects were dependent on innumerable variables such as age and physical activity. Similarly, the present study did not find any correlation between body mass, BMI, and fat mass and BMD. Low body mass has been declared to be a significant risk factor in the development of osteoporosis; on the other hand, obesity has been mentioned as a significant confounder of BMD [29].

Several reports have demonstrated a correlation between BMC and fat mass in obese children, although their results seem controversial [17, 19]. On the other hand, it seems quite clear that lean mass is the best primary predictor of BMC and BMD in obese children [6, 30]. Similar to previous reports, we showed a strong positive correlation between free fat mass, BMD and BMC.

Previous studies verified that lean body mass and fat mass had significant correlation with insulin sensitivity, it having been demonstrated that insulin enhances osteoblast proliferation and collagen production, but it is unclear if this action is impaired in patients with increased insulin resistance [31, 32]. In fact, in the present study only in girls did we see a positive correlation between FFM and insulin concentration ( $r = 0.28$ ;  $P \leq .036$ ). Studies have reported increased, decreased or unchanged BMD in diabetic and post-menopausal females compared with normal glucose tolerance [31, 33].

An interesting result observed in the present study is that insulin levels and HOMA-IR were negatively correlated with BMD. In fact, it was observed in obese adolescents with metabolic syndrome that all of them presented insulin resistance and increased visceral and hepatic fats [34, 35].

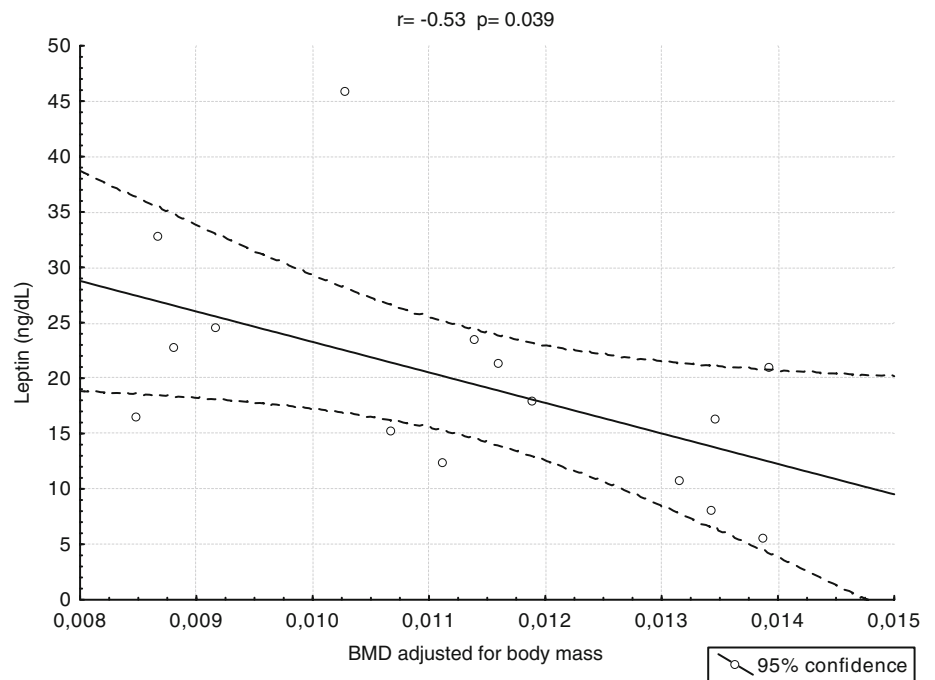
Thus, it is hypothesized that patients with insulin resistance in the metabolic pathway exhibit resistance to skeletal action, causing an impairment in the IGF-I axis modulated by this hormone, leading in the later to a low BMD as observed in obese children after puberty. However a positive correlation between BMD and the fasting insulin level and HOMA-IR was found [36].

We are in agreement with Afghani et al. [6] who have concluded that type 2 diabetes and the hyperinsulinemic state typically associated with obesity and insulin resistance in these patients could cause low body mass (independent of body size) and that this relationship is possibly mediated by deficiency in insulin like growth factors. Studies have found low, normal and high IGF-1 levels in obese subjects [37–40]. Whether or not obese children and adolescents have abnormal IGF-1 levels should be investigated in longitudinal studies of this population.

Another hypothesized underlying mechanism is that osteoporosis is common associated with various inflammatory conditions. It is known that pro-inflammatory cytokines up-regulate receptor activator of nuclear factor- $\kappa\beta$  ligand, leading to increased bone resorption and osteoporosis. C-reactive protein (CRP) is a systemic inflammatory marker regulated by cytokines such as IL-1, IL-6 and TNF- $\alpha$ . Some have suggested that an elevated CPR is associated with osteoporosis. Finally, the systemic inflammation related to a metabolic syndrome might activate bone resorption and lead to reduced BMD [41].

In a recent study it was demonstrated that, considering total body weight and lean mass of obese children and

**Fig. 1** Regression linear analysis of BMD adjusted for body mass as dependent variable in obese adolescents boys



**Table 3** Simple correlation coefficient between free fat mass and biochemical parameters in obese adolescents

	Boys (n = 41)		Girls (n = 68)	
	r	P	r	P
Free fat mass (kg)				
Insulin (uU/ml)	-0.718	0.66	0.276	0.036
HOMA-IR	-0.118	0.491	0.262	0.058
Leptin (ng/dl)	0.031	0.866	0.056	0.698

HOMA-IR Homeostasis model assessment insulin resistance index

**Table 4** Multiple regression analysis of BMD as dependent variable in obese adolescents

Variables	Model	R <sup>2</sup>	P
<b>Boys</b>			
Free fat mass (kg)	1	0.7988	0.0006
Visceral fat (cm)	2	0.8984	0.0099
BMI (kg/m <sup>2</sup> )	3	0.9144	0.2124
<b>Girls</b>			
Free fat mass (kg)	1	0.3653	0.0397
Visceral fat (cm)	2	0.4750	0.0735
Height (m)	3	0.5647	0.0602
Subcutaneous fat (cm)	4	0.6022	0.1843

BMI Body mass index

adolescents, these skeletal responses were not sufficient to compensate for the excess load on the whole body [15]. Thus, it is important to control obesity in childhood in order to prevent low BMD after puberty, showing that

**Table 5** Multiple regression analysis of BMC as dependent variable in obese adolescents

Variables	Model	R <sup>2</sup>	P
<b>Boys</b>			
Free fat mass (kg)	1	0.8150	0.0003
Insulin (uU/ml)	2	0.8986	0.0154
<b>Girls</b>			
Free fat mass (kg)	1	0.6182	0.0001
Visceral fat (cm)	2	0.6901	0.0300
BMI (kg/m <sup>2</sup> )	3	0.7031	0.3236

BMI Body mass index

multidisciplinary therapy of obesity is essential to contribute to bone health. Indeed, it was observed in obese adolescents that BMC continues to increase during weight loss and remains higher than that observed in a reference group. These results suggest that to optimize the health benefits of weight loss among obese adolescents, their bone health should be better understood and addressed [17].

A relevant study showed that physical activity is an important behavior of children’s bone development, particularly as a potentially modifiable determinant of peak bone mass, including a contribution to improving insulin levels, HOMA-IR, and free fat mass. These data reinforce that the essential role of activity’s impact on bone health is central to developing primary prevention strategies for osteoporosis [27].

The identification of leptin as a major hormonal product of the adipocyte has raised the possibility that this peptide may contribute to fat mass/bone density. However, an

association seen in human cross-sectional studies does not establish causality [1]. In this study, linear regression analysis showed in boys that serum leptin is inversely associated to BMD adjusted for body mass ( $r = -0.53$ ;  $P \leq 0.05$ ) (Fig. 1). This results are in agreement with Morberg et al. [1], who observed the same results in two groups of middle-aged men with very different weight histories in childhood and young adulthood: fasting serum leptin was negatively associated with BMD adjusted for body weight, indicating that an increase in serum leptin concentration, independent of body size, may be inversely associated with BMD.

However, Zhong et al. [42] suggested that serum leptin concentration was not a direct determinant of BMD. In human beings, positive, negative or no associations between serum leptin levels and BMD have been reported [43–45]. The relationship between leptin and bone is a complex one, with a diverging effect depending on whether central or peripheral mechanisms are operating [46].

Cross-sectional studies of the relationship between leptin and BMD in humans provide data that are not very conclusive. Thomas et al. [47], were the first to publish the hypothesis of an association between serum leptin levels and BMD in men, and concluded that fat mass and leptin are weakly and inconsistently predictive of BMD in men. However, this study did not include obese subjects.

The present study has limitations: the sample size was not large enough. However, the study has some strengths. Leptin levels in the obese subjects spanned a wider range than previously reported [10, 45], thus allowing the investigation of the association between leptin and mineral metabolism across a spectrum. Finally, all female subjects were normally cycling, which excluded any confounding effect of irregular menses on bone metabolism.

In conclusion, there is a dichotomy in the impact of body composition parameters and leptin, insulin levels and insulin resistance on bone parameters in obese Brazilian girls. Data from the present study support the hypothesis that leptin, insulin and HOMA-IR are inversely associated with BMD and play a significant direct role in bone metabolism.

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## References

- Morberg CM, Teteis I, Black E, Toubro S, Soerensen TIA, Pedersen O, Astrup A (2003) Leptin and bone mineral density: a cross-sectional study in obese and nonobese men. *J Clin Endocrinol Metab* 88:5795–5800
- Siris ES, Miller PD, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, Berger ML, Santora AC, Sherwood LM (2001) Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women: results from the National Osteoporosis Risk Assessment. *JAMA* 286:2815–2822
- Kirchengast S, Knogler W, Hauser G (2002) Protective effect of moderate overweight on bony density of the hip joint in elderly and old Austrians. *Anthropol Anz* 60:187–197
- Rico H, Arribas I, Casanova FJ, Duce AM, Hernandez ER, Cortez-Prieto J (2002) Bone mass, bone metabolism gonadal status and body mass index. *Osteoporos Int* 13:187–379
- Krakauer JC, McKenna MJ, Buderer NF, Rao DS, Whitehouse FW, Parfitt AM (1995) Bone loss and bone turnover in diabetes. *Diabetes* 44:775–782
- Afghani A, Cruz ML, Goran MI (2005) Impaired glucose tolerance and bone mineral content in overweight latino children with a family history of type 2 diabetes. *Diabetes Care* 28:372–378
- Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G (2000) Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell* 100:197–207
- Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G (2002) Leptin regulates bone formation via the sympathetic nervous system. *Cell* 111:305–317
- Zhang Y, Proença R, Maffei M, Baroni M, Lori L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432
- Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL (1999) Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology* 140:1630–1938
- Sun AJ, Jing T, Heymsfield SB, Philips GB (2003) Relationship of leptin and sex hormones to bone mineral density in men. *Acta Diabetol* 40:S101–S105
- Zoico E, Zamboni M, Adami S, Vettor R, Mazzali G, Tosoni P, Bissoli L, Bosello O (2003) Relationship between leptin levels and bone mineral density in the elderly. *Clin Endocrinol* 59:97–103
- Hamrick MW, Pennington C, Newton D, Xie D, Isales C (2004) Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone* 34:376–383
- Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS (2004) Obesity during childhood and adolescence augments bone mass and bone dimensions. *Am J Clin Nutr* 80:514–523
- Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D (2008) Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. *J Bone Miner Metab* 26:73–78
- da Silva HG, Mendonça LM, Conceição FL, Zahar SE, Farias ML (2007) Influence of obesity on bone density in postmenopausal women. *Arq Bras Endocrinol Metabol* 51:943–949
- Stettler N, Berkowitz RI, Cronquist JL, Shults J, Wadden TA, Zemel BS et al (2008) Observational study of bone accretion during successful weight loss in obese adolescents. *Obesity* 16:96–101
- Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ (2001) Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. *J Pediatr* 139:509–515
- Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM (2000) Overweight and obese children have low bone mass and area for their weight. *Int J Obes Relat Metab Disord* 24:627–632
- Tanner JM, Whitehouse RH (1976) Clinical longitudinal standards for height, weight velocity and stages of puberty. *Arch Dis Child* 51:170–179

21. Gutin B, Ransey L, Barbeau P et al (1999) Plasma leptin concentrations in obese children: changes during 4-mo periods with and without physical training. *Am J Clin Nutr* 69:388–394
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and  $\beta$ -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419
23. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ (2000) Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 85:2402–2410
24. Schwimmer JB, Deutsch R, Rauch JB, Bahling C, Newbury R, Lavine JG (2003) Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. *J Pediatr* 143:500–505
25. Black E, Petersen L, Kreutz M, Toubro S, Sorensen TI, Astrup A (2002) Fat mass measured by DXA varies with scan mode. *Obes Res* 10:69–77
26. Lorentzon M, Lorentzon R, Bäckström T, Nordström P (1999) Estrogen receptor gene polymorphism, but not estradiol levels, is related to bone density in health adolescent boys: a cross-sectional and longitudinal study. *J Clin Endocrinol Metab* 84:4597–4601
27. Janz K (2002) Physical activity and bone development during childhood and adolescence. Implications for the prevention of osteoporosis. *Minerva Pediatr* 54:93–104
28. Jüremäe T, Sööt T, Jüremäe J (2005) Relationship of anthropometrical and body composition with bone mineral content or density in young women with different levels of physical activity. *J Physiol Anthropol Appl Human Sci* 24:579–587
29. Holbrook TL, Barret-Connor E (1993) The association of lifetime weight and height control patterns with bone mineral density in an adult community. *Bone Miner* 20:141–142
30. Ackerman A, Thornton JC, Wang J, Pierson RN Jr, Horlick M (2006) Sex difference in the effect of puberty on the relationship between fat mass and bone mass in 926 healthy subjects, 6 to 18 years old. *Obesity* 14:819–825
31. Thomas DM, Ng KW, Best JD (1997) Insulin and bone: a clinical and scientific review. *Endocrinol Metab* 4:5–17
32. Abrahamsen B, Rohold A, Henriksen JE, Beck-Nielsen H (2000) Correlations between insulin sensitivity and bone mineral density in non-diabetic men. *Diabet Med* 17:124–129
33. Abou Samra R, Baba NH, Torbay N, Dib L, Fuleihan GEH (2005) High plasma leptin levels is not associated with higher bone mineral density in insulin-resistance premenopausal obese women. *J Clin Endocrinol Metab* 90:2588–2594
34. Caranti DA, Lazzer S, Dâmaso AR, Agosti F, Zennaro R, de Mello MT, Tufik S, Sartorio A (2008) Prevalence and risk factors of metabolic syndrome in Brazilian and Italian obese adolescents: a comparison study. *Int J Clin Pract* 62:1526–1532
35. Dâmaso AR, do Prado WL, de Piano A, Tock L, Caranti DA, Lofrano MC, Carnier J, Cristofalo DJ, Lederman H, Tufik S, de Mello MT (2008) Relationship between nonalcoholic fatty liver disease prevalence and visceral fat in obese adolescents. *Dig Liver Dis* 40:132–139
36. Nagasaki K, Kikuchi T, Hiura M, Uchiyama M (2004) Obese Japanese children have low bone mineral density after puberty. *J Bone Miner Metab* 22:376–381
37. Maccario M, Ramunni J, Oleandri SE, Procopio M, Grottoli S, Rossetto R, Savio P, Aimaretti G, Camanni F, Ghigo E (1999) Relationships between IGF-I and age, gender, body mass, fat distribution, metabolic and hormonal variables in obese patients. *Int J Obes Relat Metab Disord* 23:612–618
38. Radetti G, Bozzola M, Pasquino B, Paganini C, Agliandolo A, Livieri C, Barreca A (1998) Growth hormone bioactivity, insulin-like growth factors (IGFs), and IGF binding proteins in obese children. *Metabolism* 47:1490–1493
39. Argente J, Caballo N, Barrios V, Pozo J, Muñoz MT, Chowen JA, Hernández M (1997) Multiple endocrine abnormalities of the growth hormone and insulin-like growth factor axis in prepubertal children with exogenous obesity: effect of short- and long-term weight reduction. *J Clin Endocrinol Metab* 82:2076–2083
40. Nam SY, Lee EJ, Kim KR, Cha BS, Song YD, Lim SK, Lee HC, Huh KB (1997) Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. *Int J Obes Relat Metab Disord* 21:355–359
41. Kinjo M, Setoguchi S, Solomon DH (2007) Bone mineral density in adults with the metabolic syndrome: analysis in a population-based U.S. sample. *J Clin Endocrinol Metab* 92:4161–4164
42. Zhong N, Wu XP, Xu ZR, Wang AH, Luo XH, Cao XZ, Xie H, Shan PF, Liao EY (2005) Relationship of serum leptin with age, body mass index, and bone mineral density in healthy mainland Chinese women. *Clin Chim Acta* 351:161–168
43. Iwamoto I, Douchi T, Kosha S, Murakami M, Pujino T, Nagata Y (2000) Relationship between serum leptin levels and regional bone mineral density, bone metabolic markers in healthy women. *Acta Obstet Gynecol Scand* 79:1060–1064
44. Odabasi E, Azata M, Turan M, Bingol N, Yonem A, Cakir B, Kutlu M, Oxdemir IC (2000) Plasma leptin concentrations in postmenopausal women with osteoporosis. *Eur J Endocrinol* 142:170–174
45. Blum M, Harris SS, Must A, Naumova EN, Phillips SM, Rand WM, Dawson-Hughes B (2003) Leptin, body composition and bone mineral density in premenopausal women. *Calcif Tissue Int* 73:27–32
46. Reid IR, Comish J (2004) Direct actions of leptin on bone remodeling. *Calcif Tissue Int* 74:313–316
47. Thomas T, Burguera B, Melton LJ, Atkinson EJ, O'Fallon WM, Riggs BL, Khosla S (2001) Role of serum leptin and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. *Bone* 29:114–120