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ORIGINAL ARTICLE

Relationships between serum leptin levels and bone mineral parameters in school-aged children: a 3-year follow-up study

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Abstract

Leptin regulates bone cell differentiation and functions via direct and indirect actions in experimental settings. Epidemiologically, however, the impact of leptin on the regulation of bone metabolism remains unclear. While some studies have reported a positive relationship between leptin and bone mineral parameters, other studies found an inverse or no association. We analyzed data from a population-based follow-up survey of community-dwelling children in Hamamatsu, Japan, to investigate relationships between leptin levels and bone mineral parameters. Multiple regression analysis was performed. Multicollinearity was quantified using the variance infiltration factor (VIF). Among 408 children who participated in the baseline survey (at age 11.2 years), 254 (121 boys and 133 girls) completed the follow-up survey (at age 14.2 years). Leptin levels were strongly related to fat mass ($r = 0.87$ in boys, $r = 0.80$ in girls). Leptin levels at baseline were significantly ($P < 0.05$) positively related to total body less head (TBLH) areal bone mineral density (aBMD) at follow-up in girls (standardized partial regression coefficient: $\beta = 0.302$, VIF = 2.246), after adjusting for body fat percentage (%). On the other hand, leptin levels were inversely related to TBLH aBMD in boys ($\beta = -0.395$, VIF = 4.116), after adjusting for body fat mass (kg). Positive relationships between leptin levels and bone mineral parameters were observed with VIF values < 4.0, whereas inverse relationships were observed with VIF values ≥ 4.0. These findings suggest that positive relationships between leptin levels and bone mineral parameters are weak, or not always observed, due to statistical problems (i.e., multicollinearity) and other factors derived from adipose tissue.

Keywords Adipokines · Child · Densitometry · General population

Introduction

Rapid bone mineral acquisition occurs during childhood and adolescence [1–3], and most of the bone mass at multiple skeletal sites is accumulated by late adolescence [1]. There exists strong evidence that the bone status during childhood is indicative of bone status in young adulthood [3]. During childhood, weight-dependent bone loading plays an important role in establishing bone mass, and body weight-induced mechanical signaling is an essential mechanism for maintaining bone health [4]. Several hormones, including pituitary-, gonadal-, thyroid-, and adipocyte-derived hormones, are also intimately associated with bone growth and turnover [4]. Adipose tissue, the major storage site for excess energy and a component of body weight, also secretes several adipokines [5]. Thus, adipose tissue can influence bone mass through its weight-bearing and endocrine functions [4]. Leptin is an adipocyte-derived hormone, initially known for its role in energy homeostasis and regulation of energy expenditure [6]. Recently, leptin has also been suggested to regulate bone cell differentiation and functions at multiple levels, via direct actions on skeletal tissue, and indirectly thorough modulation of bone-regulating hormones in experimental settings [4, 5, 7, 8].

Currently, epidemiological evidence linking leptin to bone health is very limited, and only one longitudinal study has investigated the relationship between leptin and bone growth in healthy children [9]. The OPUS School Meal Study reported an inverse association between leptin and

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bone size after adjusting for fat mass (FM) by multivariate regression analysis [9]. On the other hand, previous cross-sectional studies have yielded conflicting results regarding the effect of leptin on bone health. Some studies found a positive relationship after adjusting for FM [10–12], while other studies showed an inverse [13, 14] or no association [15] between leptin and bone health, after adjusting for FM or body mass. These conflicting results could be explained partly by multicollinearity, which arises when two or more predictor variables in a regression analysis are moderately or highly correlated [16]. Multicollinearity causes numerical difficulties in estimating parameters, as some predictor variables supply redundant information [16]. Leptin, the product of the obese gene, is an adipocyte-secreted hormone [6] and its serum concentrations have been reported to be correlated moderately or highly with whole-body FM in a healthy, randomly selected population [17]. Thus, when leptin and FM are used in the same multiple regression model, parameter estimates may lead to incorrect interpretations about the direction and/or magnitude of predictor variable effects on bone health [16, 18]. As such, the impact of leptin on the regulation of bone metabolism has not been clarified in human studies.

In the present study, we analyzed data from a 3-year follow-up survey of school-aged healthy children in Hamamatsu, Japan, to investigate relationships between serum leptin levels and bone mineral parameters. In addition, we used several regression models to evaluate the severity of multicollinearity.

Materials and methods

Subjects

The source population (accessible population) of the baseline survey (at age 11) consisted of all fifth grade public schoolchildren who were registered in Aritama Elementary School and Sekishi Elementary School in Hamamatsu City, Japan, in November or December in 2010 and 2011. A total of 521 students (268 boys and 253 girls) were enrolled in these two schools at the time of the survey. Since there were no other private or public elementary schools in this area, most children living in the area were enrolled in either one of these two schools. The baseline survey was conducted at each elementary school.

Most children go on to Sekishi Junior High School, which is the only junior high school in the area. Accordingly, the 3-year follow-up survey (at age 14) was conducted at this junior high school in December 2013 and 2014. Of the source population, baseline data were obtained from 408 children (202 boys and 206 girls), and follow-up data were obtained from 254 children (121 boys and 133 girls). Thus, the present study population comprised a total of 254 students.

Prior to baseline and follow-up surveys, all parents and guardians of the students received printed information regarding the study and provided us with their written consent. The printed information included the dose of radiation exposure from dual-energy X-ray absorptiometry (DXA). All students were also allowed to decline participation on their own accord. This study was performed in accordance with the ethical standards set forth in the Declaration of Helsinki and approved by the Ethics Committee of the Kindai University Faculty of Medicine.

Body fat and bone mineral measurements

Body fat and bone mineral measurements were performed using a single DXA scanner (QDR-4500A, Hologic Inc., Bedford, MA, USA) mounted on a mobile examination car in each school at both baseline and follow-up surveys. An experienced radiological technologist performed all scans and scan analyses. Quality control of the DXA scanner was performed using the Step Phantom scan throughout the surveys. Subjects wore light clothing without metal objects while undergoing whole-body scanning. FM (kg), body fat percentage (FM divided by body mass, %), and height-normalized index of FM (fat mass index; FM divided by height squared, kg/m^2 [19, 20]) were measured. In scan analyses, total body less head (TBLH) areal bone mineral density (aBMD) and TBLH bone mineral content (BMC) were measured according to the revised 2013 International Society for Clinical Densitometry Pediatric Official Positions [21]. In addition, bone parameters of appendicular and lumbar spine regions were obtained. The head region was separated by the neck line, and the lumbar spine region was detected by the T12–L1 horizontal line and upper pelvic line in the anterior scan image using standard manufacturer-recommended procedures [22]. Furthermore, the arms were separated from the trunk by a vertical shoulder line passing through the humeral head at the glenoid fossa, and the legs were separated from the trunk by angled lines that defined a pelvic triangle and bisected both femoral necks [22]. Appendicular BMC and bone area were calculated as the sum of arm and leg bone parameters. Bone mineral apparent density (BMAD) was calculated as an estimate of volumetric BMD for children and adolescent using the following formulae: lumbar spine BMAD = lumbar spine BMC/lumbar spine area$^{3/2}$ and total body (TB) BMAD = TB BMC/(TB area^2/body height) [23].

Anthropometric measurement and blood analysis

Body weight and height were measured in light clothing with no shoes at the time of DXA measurements. Body mass

index (kg/m^2) was calculated by dividing body weight by height squared. To determine overweight and underweight subjects, international cutoffs of body mass index for adults of 25 and 18.5 kg/m^2, respectively, were used. Cutoffs for overweight boys and girls have been reported to be > 20.55 and > 20.74 kg/m^2, respectively [24], and for underweight boys and girls, < 14.97 and < 15.05 kg/m^2, respectively [25].

Blood samples were obtained by vein puncture at the time of baseline surveys, and all serum samples were stored at − 80 °C until analysis. Serum leptin levels were measured with a commercially available kit (Quantikine® Human Leptin, R&D Systems, Inc., Minneapolis, MN, USA). The intra- and inter-assay coefficients of variation were 2.0 and 5.2%, respectively.

Statistical analysis

Differences between the data of the boys and those of the girls were evaluated using the unpaired *t* test or Mann–Whitney *U* test. Sex-specific differences between the study population and the dropout population were also evaluated using the unpaired *t* test or Mann–Whitney *U* test. Pearson's correlation test was used to assess relationships between leptin levels and body fat variables. To evaluate relationships between leptin levels and bone mineral parameters, and between body fat and bone mineral parameters, simple regression analysis was used. Multiple regression analysis was used to assess relationships between leptin levels and bone variables. Height was incorporated into all regression models of BMC [26]. Multicollinearity was quantified using the variance infiltration factor (VIF), which was defined as the inverse of tolerance. A VIF > 4.0 was considered an indication of harmful multicollinearity in a regression model [16, 27]. $P < 0.05$ was considered to be statistically significant. All analyses were performed using SPSS Statistics Desktop for Japan, Version 22 (IBM Japan, Ltd., Tokyo, Japan).

Results

Table 1 shows sex-specific differences between the study population and the dropout population. Except for the percentage of overweight girls, there were no significant differences in subject characteristics between the two populations. Table 2 shows relationships between leptin levels and body fat variables at baseline. Serum leptin levels had significant strong relationships with all fat variables. Table 3 shows relationships between leptin levels at baseline and bone mineral parameters, and between body fat at baseline and bone mineral parameters. There were significant weak to moderate relationships between FM and bone mineral parameters. Serum leptin levels also showed significant very weak to weak relationships with bone mineral parameters.

Table 4 shows the results of cross-sectional analysis on the relationships between leptin levels and bone mineral parameters at baseline. Leptin levels were significantly positively related to appendicular aBMD, lumbar spine aBMD, and TBLH aBMD in Model 2 for girls, and to TB BMAD in Model 1 for girls. All models had VIF values < 4.0. In contrast, leptin levels were significantly inversely related to lumbar spine aBMD and BMC in Model 1 for boys, with a VIF value of ≥ 4.0.

Table 5 shows the results of longitudinal analysis on the relationships between leptin levels at baseline and bone mineral parameters at follow-up. Leptin levels were significantly positively related to appendicular aBMD, lumbar spine BMC, TBLH aBMD, and TBLH BMC in Model 2 for girls, with a VIF value of < 4.0. In contrast, leptin levels were significantly inversely related to lumbar spine BMAD, lumbar spine aBMD, and TBLH aBMD in Model 1 for boys, with a VIF value of ≥ 4.0.

Discussion

This prospective cohort study investigated relationships between serum leptin levels and bone mineral parameters in school-aged children in Japan. Our longitudinal analysis revealed conflicting results in terms of the direction of the effect of leptin levels on bone mineral parameters. When leptin and FM were included in the same multiple regression models, some regression models showed a positive relationship, while others showed an inverse or no relationship between serum leptin levels and bone mineral parameters. Interestingly, positive relationships were observed with VIF values < 4.0, whereas inverse relationships were observed with VIF values ≥ 4.0. These results suggest that the direction of the effect of leptin levels on bone mineral parameters could change from model to model, as the methods of adjustment for FM differ in each multiple linear regression model. Thus, the observed inverse relationships with VIF values ≥ 4.0 may be attributed to multicollinearity, and may not represent a correct direction of the effect of leptin levels on bone mineral parameters [16]. Our results suggest that, to assess relationships between leptin levels and bone mineral parameters independent of FM, an evaluation of multicollinearity in multiple regression analysis is necessary.

Other than the OPUS School Meal Study [9] and the present study, no longitudinal studies have been conducted in healthy school-aged children with follow-up data on leptin and bone growth. The OPUS School Meal Study reported that leptin was inversely associated with bone size after adjusting for FM [9]. However, since the regression models used in that study were not assessed based on VIF values, the

Table 1 Baseline characteristics of the study population and dropout population

	Study population			Dropout population		Difference between study and dropout populations[a]	
	Boys ($N = 121$)	Girls ($N = 133$)	Difference between boys and girls[a]	Boys ($N = 81$)	Girls ($N = 73$)	Boys	Girls
Age (years)	11.2 ± 0.3	11.2 ± 0.3	ns	11.2 ± 0.3	11.2 ± 0.3	ns	ns
Height (cm)	141 ± 6	143 ± 7	0.007	142 ± 6	143 ± 7	ns	ns
Weight (kg)	34.7 ± 7.4	35.1 ± 5.9	ns	35.4 ± 7.1	35.5 ± 8.7	ns	ns
Body mass index (kg/m^2)	17.3 ± 2.7	17.0 ± 1.9	ns	17.4 ± 2.5	17.1 ± 2.9	ns	ns
Overweight[b], *N* (%)	13 (11)	5 (4)	0.031	12 (15)	8 (11)	ns	0.043
Underweight[b], *N* (%)	18 (15)	16 (12)	ns	9 (11)	13 (18)	ns	ns
Fat mass (kg)	7.1 ± 3.8	7.7 ± 2.6	ns	7.4 ± 3.5	7.8 ± 4.0	ns	ns
Body fat percentage (%)	18.9 ± 6.1	20.9 ± 4.5	0.004	19.6 ± 5.9	20.7 ± 4.8	ns	ns
Fat mass index (kg/m^2)	3.5 ± 1.7	3.7 ± 1.1	ns	3.6 ± 1.6	3.7 ± 1.6	ns	ns
Leptin level (ng/mL)	2.5 ± 3.2	3.4 ± 2.6	0.011	2.5 ± 2.7	3.8 ± 3.6	ns	ns
Appendicular aBMD (g/cm^2)	0.65 ± 0.04	0.66 ± 0.05	ns	0.65 ± 0.04	0.65 ± 0.05	ns	ns
Appendicular BMC (g)	430 ± 97	460 ± 104	0.018	443 ± 83	453 ± 122	ns	ns
Lumbar spine BMAD (g/cm^3)	0.10 ± 0.01	0.10 ± 0.01	0.027	0.10 ± 0.01	0.10 ± 0.01	ns	ns
Lumbar spine aBMD (g/cm^2)	0.58 ± 0.05	0.65 ± 0.08	< 0.001	0.59 ± 0.05	0.64 ± 0.09	ns	ns
Lumbar spine BMC (g)	22 ± 7	28 ± 9	< 0.001	24 ± 5	28 ± 8	ns	ns
TBLH aBMD (g/cm^2)	0.61 ± 0.04	0.62 ± 0.05	0.005	0.61 ± 0.04	0.62 ± 0.06	ns	ns
TBLH BMC (g)	644 ± 140	700 ± 162	0.003	656 ± 121	690 ± 178	ns	ns
TB BMAD (g/cm^3)	0.08 ± 0.01	0.08 ± 0.01	ns	0.08 ± 0.01	0.08 ± 0.01	ns	ns

Values represent mean ± standard deviation, or *N* (percentage)

Fat mass index was calculated as fat mass divided by height squared

N number, *TBLH* total body less head, *aBMD* areal bone mineral density, *BMC* bone mineral content, *ns* not significant

[a]*P* value calculated with the unpaired *t* test or Mann–Whitney *U* test

[b]International body mass index cutoffs were used to determine overweight and underweight children

Table 2 Relationships between leptin levels and body fat variables at baseline

	Boys ($N = 121$)		Girls ($N = 133$)	
	r	*P*	*r*	*P*
Fat mass	0.870	< 0.001	0.795	< 0.001
Body fat percentage	0.816	< 0.001	0.745	< 0.001
Fat mass index	0.878	< 0.001	0.803	< 0.001

Fat mass index was calculated as fat mass divided by height squared

N number, *r* Pearson's correlation coefficient

influence of multicollinearity is unclear. In the present study, we found significant positive relationships between leptin levels and bone mineral parameters in girls after adjusting for body fat percentage, with a VIF value of < 4.0. However, we also found no significant relationship between leptin levels and BMC after adjusting for fat variables, where VIF values were < 4.0. One possible reason for the negative (i.e., not significant) results after adjusting for body fat is that the weight-induced mechanical effects of FM on bone mass might be stronger than the hormonal effects of leptin, which is secreted from adipocytes (FM). Leptin is the product of the obese gene in adipose tissue [6], and indeed, strong positive relationships were observed between leptin levels and body fat variables in the present study. Our results infer that the strong effects of mechanical signal of FM on bone mass might obscure the effects of leptin on bone mass, and that the significant relationship between leptin and bone mass might consequently disappear, when adjusted for FM, in statistical analyses. Mechanical signaling by weight-dependent loading of FM on bone may be an essential mechanism for bone growth [4, 28].

On the other hand, adipose tissue can influence bone growth due to weight-bearing loading and many endocrine mediators which are secreted from adipocytes [4]. The chemical bone–fat connections consist of inflammatory cytokines (e.g., interleukin-1, interleukin-6, and tumor necrosis factor-α) released by adipocytes that directly target bone and adipokines (e.g., leptin and adiponectin) that

Table 3 Relationships between leptin levels and bone mineral parameters, and between body fat and bone mineral parameters

	Leptin levels at baseline				Fat mass at baseline				Body fat percentage at baseline			
	Boys ($N = 121$)		Girls ($N = 133$)		Boys ($N = 121$)		Girls ($N = 133$)		Boys ($N = 121$)		Girls ($N = 133$)	
	β	P	β	P	β	P	β	P	β	P	β	P
Baseline												
Appendicular aBMD	0.140	ns	0.240	0.005	0.220	0.015	0.356	< 0.001	0.070	ns	0.062	ns
Appendicular BMC	0.391	< 0.001	0.358	< 0.001	0.587	< 0.001	0.595	< 0.001	0.392	< 0.001	0.263	0.002
Lumbar spine BMAD	0.156	ns	0.110	ns	0.085	ns	0.121	ns	0.177	ns	0.150	ns
Lumbar spine aBMD	− 0.024	ns	0.126	ns	0.143	ns	0.283	0.001	− 0.005	ns	− 0.018	ns
Lumbar spine BMC	− 0.091	ns	0.087	ns	0.117	ns	0.244	0.005	− 0.065	ns	− 0.082	ns
TBLH aBMD	0.157	ns	0.229	0.008	0.274	0.002	0.373	< 0.001	0.119	ns	0.060	ns
TBLH BMC	0.342	< 0.001	0.319	< 0.001	0.549	< 0.001	0.549	< 0.001	0.349	< 0.001	0.203	0.019
TB BMAD	− 0.452	< 0.001	− 0.386	< 0.001	− 0.662	< 0.001	− 0.601	< 0.001	− 0.525	< 0.001	− 0.412	< 0.001
Follow-up												
Appendicular aBMD	0.114	ns	0.163	ns	0.226	0.013	0.273	0.001	0.088	ns	0.051	ns
Appendicular BMC	0.277	0.002	0.177	0.041	0.468	< 0.001	0.388	< 0.001	0.302	< 0.001	0.110	ns
Lumbar spine BMAD	0.127	ns	0.053	ns	0.258	0.004	0.105	ns	0.178	ns	0.019	ns
Lumbar spine aBMD	0.145	ns	0.088	ns	0.334	< 0.001	0.203	0.019	0.190	0.037	− 0.020	ns
Lumbar spine BMC	0.130	ns	0.086	ns	0.319	< 0.001	0.213	0.014	0.168	ns	− 0.061	ns
TBLH aBMD	0.161	ns	0.161	ns	0.295	0.001	0.273	0.002	0.157	ns	0.036	ns
TBLH BMC	0.246	0.007	0.171	0.050	0.447	< 0.001	0.374	< 0.001	0.281	0.002	0.080	ns
TB BMAD	− 0.250	0.006	0.026	ns	− 0.392	< 0.001	− 0.079	ns	− 0.334	< 0.001	− 0.060	ns

Simple regression analysis was used to assess relationships

N number, β standardized partial regression coefficient, *ns* not significant, *aBMD* areal bone mineral density, *BMAD* bone mineral apparent density, *BMC* bone mineral content, *TB* total body, *TBLH* total body less head

$P < 0.05$ was considered statistically significant

Table 4 Relationships between leptin levels at baseline and bone mineral parameters at baseline

Dependent variable	Independent variables		Boys ($N = 121$)			Girls ($N = 133$)		
			β	P	VIF	β	P	VIF
Appendicular aBMD								
	Model 1	Leptin levels	− 0.213	ns	4.116	− 0.117	ns	2.717
		Fat mass	0.406	0.027	4.116	0.449	0.001	2.717
	Model 2	Leptin levels	0.248	ns	2.995	0.434	< 0.001	2.246
		Body fat percentage	− 0.133	ns	2.995	− 0.261	0.040	2.246
Appendicular BMC								
	Model 1	Leptin levels	− 0.105	ns	4.609	− 0.078	ns	2.865
		Fat mass	0.403	< 0.001	5.352	0.355	< 0.001	3.354
		Height	0.646	< 0.001	1.370	0.697	< 0.001	1.297
	Model 2	Leptin levels	0.135	ns	3.008	0.113	ns	2.352
		Body fat percentage	0.110	ns	3.017	0.094	ns	2.264
		Height	0.738	< 0.001	1.062	0.798	< 0.001	1.059
Lumbar spine BMAD								
	Model 1	Leptin levels	0.336	ns	4.116	0.037	ns	2.717
		Fat mass	− 0.207	ns	4.116	0.092	ns	2.717
	Model 2	Leptin levels	0.033	ns	2.995	− 0.004	ns	2.246
		Body fat percentage	0.150	ns	2.995	0.153	ns	2.246
Lumbar spine aBMD								
	Model 1	Leptin levels	− 0.610	< 0.001	4.116	− 0.269	ns	2.717
		Fat mass	0.674	< 0.001	4.116	0.496	< 0.001	2.717
	Model 2	Leptin levels	− 0.059	ns	2.995	0.312	0.017	2.246
		Body fat percentage	0.043	ns	2.995	− 0.250	ns	2.246
Lumbar spine BMC								
	Model 1	Leptin levels	− 0.468	0.003	4.609	− 0.054	ns	2.865
		Fat mass	0.292	ns	5.352	− 0.016	ns	3.354
		Height	0.543	< 0.001	1.370	0.701	< 0.001	1.297
	Model 2	Leptin levels	− 0.181	ns	3.008	0.118	ns	2.352
		Body fat percentage	− 0.061	ns	3.017	− 0.243	0.011	2.264
		Height	0.618	< 0.001	1.062	0.682	< 0.001	1.059
TBLH aBMD								
	Model 1	Leptin levels	− 0.337	ns	4.116	− 0.182	ns	2.717
		Fat mass	0.568	0.002	4.116	0.518	< 0.001	2.717
	Model 2	Leptin levels	0.177	ns	2.995	0.415	0.001	2.246
		Body fat percentage	− 0.025	ns	2.995	− 0.249	0.050	2.246
TBLH BMC								
	Model 1	Leptin levels	−0.164	ns	4.609	− 0.075	ns	2.865
		Fat mass	0.411	< 0.001	5.352	0.294	< 0.001	3.354
		Height	0.657	< 0.001	1.370	0.728	< 0.001	1.297
	Model 2	Leptin levels	0.088	ns	3.008	0.121	ns	2.352
		Body fat percentage	0.102	ns	3.017	0.027	ns	2.264
		Height	0.751	< 0.001	1.062	0.809	< 0.001	1.059
TB BMAD								
	Model 1	Leptin levels	0.513	< 0.001	4.116	0.251	0.028	2.717
		Fat mass	− 1.108	< 0.001	4.116	− 0.801	< 0.001	2.717
	Model 2	Leptin levels	−0.069	ns	2.995	− 0.177	ns	2.246
		Body fat percentage	− 0.469	< 0.001	2.995	− 0.281	0.020	2.246

Multiple regression analysis was used to assess relationships

N number, β standardized partial regression coefficient, *VIF* variance inflation factor, *ns* not significant, *aBMD* areal bone mineral density, *BMAD* bone mineral apparent density, *BMC* bone mineral content, *TB* total body, *TBLH* total body less head

$P < 0.05$ was considered statistically significant

Table 5 Relationships between leptin levels at baseline and bone mineral parameters at follow-up

Dependent variable	Independent variables		Boys ($N = 121$)			Girls ($N = 133$)		
			β	P	VIF	β	P	VIF
Appendicular aBMD								
	Model 1	Leptin levels	− 0.340	ns	4.116	− 0.148	ns	2.717
		Fat mass	0.522	0.004	4.116	0.391	0.005	2.717
	Model 2	Leptin levels	0.127	ns	2.995	0.280	0.031	2.246
		Body fat percentage	− 0.015	ns	2.995	− 0.158	ns	2.246
Appendicular BMC								
	Model 1	Leptin levels	− 0.177	ns	4.547	− 0.139	ns	2.863
		Fat mass	0.404	0.003	5.051	0.351	0.001	3.036
		Height at follow-up	0.618	< 0.001	1.261	0.600	< 0.001	1.122
	Model 2	Leptin levels	0.122	ns	2.998	0.164	ns	2.252
		Body fat percentage	0.048	ns	3.065	− 0.039	ns	2.247
		Height at follow-up	0.701	< 0.001	1.052	0.668	< 0.001	1.004
Lumbar spine BMAD								
	Model 1	Leptin levels	− 0.401	0.025	4.116	− 0.082	ns	2.717
		Fat mass	0.608	< 0.001	4.116	0.171	ns	2.717
	Model 2	Leptin levels	− 0.053	ns	2.995	0.088	ns	2.246
		Body fat percentage	0.220	ns	2.995	− 0.046	ns	2.246
Lumbar spine aBMD								
	Model 1	Leptin levels	− 0.599	< 0.001	4.116	− 0.198	ns	2.717
		Fat mass	0.855	< 0.001	4.116	0.360	0.011	2.717
	Model 2	Leptin levels	− 0.030	ns	2.995	0.231	ns	2.246
		Body fat percentage	0.214	ns	2.995	− 0.191	ns	2.246
Lumbar spine BMC								
	Model 1	Leptin levels	− 0.273	ns	4.547	− 0.067	ns	2.863
		Fat mass	0.357	0.023	5.051	0.158	ns	3.036
		Height at follow-up	0.567	< 0.001	1.261	0.438	< 0.001	1.122
	Model 2	Leptin levels	0.008	ns	2.998	0.261	0.023	2.252
		Body fat percentage	0.020	ns	3.065	− 0.274	0.017	2.247
		Height at follow-up	0.642	< 0.001	1.052	0.467	< 0.001	1.004
TBLH aBMD								
	Model 1	Leptin levels	− 0.395	0.026	4.116	− 0.151	ns	2.717
		Fat mass	0.639	< 0.001	4.116	0.393	0.005	2.717
	Model 2	Leptin levels	0.099	ns	2.995	0.302	0.021	2.246
		Body fat percentage	0.076	ns	2.995	− 0.189	ns	2.246
TBLH BMC								
	Model 1	Leptin levels	− 0.230	ns	4.547	− 0.134	ns	2.863
		Fat mass	0.431	0.002	5.051	0.337	0.003	3.036
		Height at follow-up	0.614	< 0.001	1.261	0.582	< 0.001	1.122
	Model 2	Leptin levels	0.082	ns	2.998	0.200	0.043	2.252
		Body fat percentage	0.059	ns	3.065	− 0.094	ns	2.247
		Height at follow-up	0.702	< 0.001	1.052	0.648	< 0.001	1.004
TB BMAD								
	Model 1	Leptin levels	0.374	0.028	4.116	0.241	ns	2.717
		Fat mass	− 0.717	< 0.001	4.116	− 0.270	ns	2.717
	Model 2	Leptin levels	0.067	ns	2.995	0.159	ns	2.246
		Body fat percentage	− 0.389	0.011	2.995	− 0.179	ns	2.246

Multiple regression analysis was used to assess relationships

N number, β standardized partial regression coefficient, *VIF* variance inflation factor, *ns* not significant, *aBMD* areal bone mineral density, *BMAD* bone mineral apparent density, *BMC* bone mineral content, *TB* total body, *TBLH* total body less head

$P < 0.05$ was considered to be statistically significant

regulate the central nervous system and thereby change the sympathetic impulses to bone [29, 30]. A recent study by Ho-Pham et al. [18] investigated the role of leptin in mediating the relationship between FM and bone mineral density in a cross-sectional study and found no significant mediated effect of leptin on bone mineral density. The contribution of weight-dependent FM loading itself, as well as other factors derived from adipose tissue on bone mass, may be greater than that of leptin. However, the authors did not rule out the impact of regulation of bone mass by leptin [18].

The present single-center, population-based, prospective cohort study has several strengths. First, a single-center study is free from inter-center variation. Moreover, a single radiological technologist performed all scans and scan analyses using a single DXA scanner. Second, the present population-based study reflects the health status of community-dwelling children in Japan. Third, the time sequence of exposure (leptin at baseline) and outcome (bone at follow-up) in the cohort design strengthens the process inferring the cause–effect relationship between leptin levels and bone mineral acquisition. Measuring serum leptin levels before bone mineral acquisition occurs helps establish the time sequence of variables. In other words, while the effect–cause relationship is often a problem in cross-sectional studies, it is less problematic in prospective cohort studies. On the other hand, there are also several limitations worth noting. First, follow-up data were obtained from 62.3% of participants of the baseline survey. The loss of subjects to follow-up might have resulted in self-selection bias. The percentage of overweight children among girls who dropped out was indeed higher than that among girls who were followed up, whereas no significant differences were observed in other baseline characteristics between dropout and follow-up subjects. This potential bias might have led to over- or underestimation of the relationships of interest. Nonetheless, the anthropometric values of our study population at baseline showed similar characteristics to those of the corresponding general population (i.e., Japanese children at age 11.2 years) as reported by a Japanese national survey (e.g., mean height in boys and girls, 142.5 and 144.0 cm, respectively; mean weight in boys and girls, 36.6 and 37.3 kg, respectively [31]). Second, we did not obtain any information about Tanner scale, physical activity, and nutrient intake, which have significant relationships with bone mineral parameters.

In conclusion, we found positive relationships between serum leptin levels at age 11 years and bone mineral parameters at age 14 years, independent of body fat percentage, in school-aged healthy children. However, given the strong relationship between leptin and body fat, associations between leptin levels and bone mineral parameters may not always be observed due to statistical problems (i.e., multicollinearity) and other factors derived from adipose tissue.

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Compliance with ethical standards

Conflict of interest All authors have no conflict of interest for the present study.

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