Author(s): Gil-Cosano, José J.; Gracia-Marco, Luis; Ubago-Guisado, Esther; Migueles, Jairo H.; Courteix, Daniel; Labayen, Idoia; Plaza-Florido, Abel; Molina-García, Pablo; Dutheil, Frédéric; Ortega, Francisco B.

Title: Leptin levels were negatively associated with lumbar spine bone mineral content in children with overweight or obesity

Year: 2022

Version: Published version

Gil-Cosano, J. J., Gracia-Marco, L., Ubago-Guisado, E., Migueles, J. H., Courteix, D., Labayen, I., Plaza-Florido, A., Molina-García, P., Dutheil, F., & Ortega, F. B. (2022). Leptin levels were negatively associated with lumbar spine bone mineral content in children with overweight or obesity. Acta Paediatrica, 111(10), 1966-1973. https://doi.org/10.1111/apa.16456

Received: 24 January 2022 | Revised: 14 June 2022 | Accepted: 15 June 2022

DOI: 10.1111/apa.16456

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

ORIGINAL ARTICLE

Leptin levels were negatively associated with lumbar spine bone mineral content in children with overweight or obesity

José J. Gil-Cosano[1,2] | Luis Gracia-Marco[1,3] | Esther Ubago-Guisado[1,3,4,5] | Jairo H. Migueles[1,6] | Daniel Courteix[7] | Idoia Labayen[8,9,10] | Abel Plaza-Florido[1] | Pablo Molina-García[1,11] | Frédéric Dutheil[12] | Francisco B. Ortega[1,6,13]

[1]PROFITH Research Group, Sport and Health University Research Institute, Department of Physical Education and Sports, Faculty of Sport Sciences, University of Granada, Granada, Spain

[2]Faculty of Health Sciences, Isabel I University, Burgos, Spain

[3]Instituto de Investigación Biosanitaria, ibs.Granada, Granada, Spain

[4]Escuela Andaluza de Salud Pública, Granada, Spain

[5]Epidemiology and Control of Chronic Diseases, CIBER of Epidemiology and Public Health (CIBERESP), Madrid, Spain

[6]Department of Biosciences and Nutrition, Karolinska Institute, Karolinska, Sweden

[7]Laboratory AME2P, CRNH Auvergne, Clermont Auvergne University, Clermont-Ferrand, France

[8]Institute for Sustainability & Food Chain Innovation, ISFOOD, University of Navarra, Pamplona, Spain

[9]Navarra Institute for Health Research, IdiSNA, Pamplona, Spain

[10]Department of Health Sciences, Public University of Navarra, Campus de Arrosadia Pamplona, Pamplona, Spain

[11]Biohealth Research Institute, ibs.Granada, Physical Medicine and Rehabilitation Service, Virgen de las Nieves University Hospital, Granada, Spain

[12]CHU Clermont-Ferrand, Preventive and Occupational Medicine, Clermont-Ferrand, France Clermont Auvergne University, CNRS, LaPSCo Physiological and Psychosocial Stress, Clermont-Ferrand, France

[13]Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland

Correspondence
Luis Gracia-Marco, PROFITH Research Group, Sport and Health University Research Institute Department of Physical Education and Sports, Faculty of Sport Sciences University of Granada, 18071 Granada, Spain.
Email:lgracia@ugr.es

Funding information
This study was mainly supported by grants from the Spanish Ministry of Economy and Competitiveness (DEP2013-47540, DEP2016-79512-R, DEP2017-91544-EXP and RYC-2011-09011), European Regional Development Fund (ERDF) of the European Commission (No 667302) and the Alicia Koplowitz Foundation. Additional funding was obtained from the Andalusian Operational Programme supported with ERDF (FEDER in Spanish,

Abstract

Aim: Adipokines seem to play a role in bone morphogenesis, although this also depends on the mechanical forces applied to the skeleton. The aim was to assess the relationships of resting leptin and adiponectin with bone parameters and whether high muscular fitness levels affect these relationships in children with overweight or obesity.

Methods: This cross-sectional study took part from 2014 to 2016 in Granada, Spain. Participants were recruited from University Hospitals, and we also used advertisements in local media and school contacts in the city. Adipokines were analysed in plasma. Muscular fitness was assessed by one repetition maximum in bench and leg press tests. Dual-energy X-ray absorptiometry was used to measure bone parameters.

Results: We included 84 children (10.0 ± 1.2y; 63% boys) in this analysis. Leptin was negatively associated with lumbar spine bone mineral content ($\beta = -0.162$, $p = 0.053$).

Abbreviations: aBMD, bone mineral density; BMC, bone mineral content; LS, lumbar spine; PHV, peak height velocity; RM, repetition maximum; TBLH, total body less head.

Based on a PhD thesis conducted at the University of Granada, Spain. Some content was presented at the European Congress of Sport Science, 28-30 October 2020, Seville, Spain.

B-CTS-355-UGR18). Additional support was obtained from University of Granada, Plan Propio de Investigación 2016, Excellence actions: Units of Excellence, Unit of Excellence on Exercise and Health, the Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades (SOMM17/6107/UGR); the EXERNET Research Network on Exercise and Health in Special populations (DEP2005-00046/ACTI); and the HL-PIVOT network - Healthy Living for Pandemic Event Protection. Funding for open access charge: Universidad de Granada / CBUA.

No significant interaction was found for muscular fitness. Simple slope estimates suggested that children performing more than 133.3 kg in leg press test ameliorated the negative association between leptin and lumbar spine bone mineral content.

Conclusion: Leptin levels were negatively associated with lumbar spine bone mineral content in children with overweight or obesity. A high muscular fitness at the lower body could counteract this association.

KEYWORDS
adipokines, bone, muscle strength, obesity, prepubertal

1 | INTRODUCTION

Bone accrual during childhood is crucial in protecting against osteoporosis later in life.[1] A growing body of evidence suggests that children with overweight or obesity tend to have higher bone parameters than their normal-weight peers.[2] However, this positive effect seems to be explained by an increase in lean mass rather than fat mass.[3] Indeed, excessive fat mass accumulation may have a negative impact on bone which may be related to the associated adverse metabolic environment.[4]

In addition to genetic and lifestyle factors, the bones of children with overweight or obesity are affected by adipokines secreted by the fat mass, including leptin and adiponectin.[5] Leptin is a 167 amino acid adipokine that promotes satiety and energy expenditure by sending signals to receptors within the hypothalamus.[6] Likewise, high leptin levels may decrease bone mass by suppressing bone formation or increasing expression of the receptor of NF-κB ligand activator which promotes bone resorption.[7] Adiponectin is a 244 amino acid adipokine that improves insulin sensitivity, thereby regulating lipid and glucose metabolism.[8] Furthermore, adiponectin affects bone metabolism through promoting osteoblastogenesis and inhibiting osteoclastogenesis, although these mechanisms might be dysregulated by sex hormones.[9]

Inconsistent associations between leptin and bone parameters have been reported in children and adolescents.[10–12] Several studies have found a positive association between leptin levels and bone mineral characteristics in children affected by obesity.[10] However, other studies reported no association in adolescent girls[11] and boys[12] after controlling for potential confounders, such as age, lean mass, fat mass and sexual maturation. With regards to adiponectin, the relationship with bone in children is also controversial.[11–13] Various studies reported no association between adiponectin levels and bone parameters in girls[11] and boys,[12] although Sayers et al.[13] showed that adiponectin levels were associated with lower bone mass in 2495 boys and 2432 girls even after controlling for lean mass, fat mass and height.

Optimal muscular fitness levels during childhood and adolescence are paramount for future bone health.[14] In this regard, previous cross-sectional studies reported that the association between muscular fitness and bone parameters was explained by lean mass in different growth stages.[15] Furthermore, scientific evidence highlights that muscular fitness is negatively associated with leptin in children, whereas no association was found with adiponectin.[16,17] Thus, muscular fitness may have an important role in the relationship between adipokines and bone parameters.

Given the conflicting evidence regarding relationship of adipokines with bones in the peri-puberal years, this study aimed to examine the association of resting leptin and adiponectin with bone parameters in children with overweight or obesity. Furthermore, this study investigated whether high muscular fitness levels counteract these relationships. We hypothesised that the concentration of adipokines would play a negative role on bone parameters, and that high muscular fitness would counteract this relationship in children with overweight or obesity.

Key notes

- Children with overweight or obesity usually have high bone density and adipokines seem to play a role in bone parameters.
- This study investigated the associations of leptin and adiponectin with bone parameters, and whether high muscular fitness levels affect these relationships in children with overweight or obesity.
- Leptin levels were negatively associated with lumbar spine bone mineral content. Moreover, muscular fitness at the lower body may counteract this association.

2 | METHODS

2.1 | Study design and participants

This cross-sectional study used baseline data from the ActiveBrains project. The trial protocol has previously been described.[18] Briefly, the ActiveBrains project is a randomised controlled trial aimed at

examining the effects of a physical exercise programme on brain and cognition, as well as on physical and mental health outcomes in children with overweight or obesity. We included pre-puberal children with overweight or obesity aged 8–11 years not having any physical disabilities or neurological disorder that affects their physical performance, and, in the case of girls, not having started the menstruation at baseline assessments. Left-handedness participants, with a score in the Attention-Deficit Hyperactivity Disorder rating scale IV above the 85th percentile or with a psychiatric diagnosis at baseline, were excluded. Of the 110 participants enrolled, the 20% was recruited in the Paediatric Unit of the San Cecilio and Virgen de las Nieves University Hospitals, Granada, Spain. In addition, we used advertisements in local media and school contacts in the city to recruit the remaining participants.

In this cross-sectional study, we included 84 children (10.0 ± 1.2 years old; 63% boys) with complete data on adipokines, namely circulating leptin and adiponectin, muscular fitness, bone parameters. The baseline data collection was divided into three waves and took part from 2014 to 2016 between the months of November to February.

A participant information sheet was given to the parents or legal guardians and a written informed consent was obtained. The ActiveBrains project was approved by the Ethics Committee on Human Research of the University of Granada (Reference: 848, February 2014), and was registered at ClinicalTrials.gov (NCT02295072).

2.2 | Anthropometry and somatic maturation

The body mass was measured to the nearest 0.1 kg using a SECA 861 electronic scale (SECA Corp). The height was measured to the nearest 0.1 cm using a SECA 225 precision stadiometer (SECA Corp). The body mass index was calculated as body mass/height (kg/m^2) and the participants were classified as children with overweight or obesity according to sex-specific and age-specific cut-off points defined by Cole et al.[19] The years from peak height velocity was used as maturational landmark and was predicted through age and anthropometric measures (height in girls and sitting height in boys) using validated algorithms for boys and girls.[20]

2.3 | Blood analyses

Blood samples were obtained between 8 am and 10 am by venepuncture after an overnight fast. The blood samples were drawn into tubes with ethylenediaminetetraacetic acid and was spun immediately (1000 × g for 10 min at 4°C). Plasma was isolated, aliquoted and stored at −80°C in the Centre of Biomedical Research in Granada, Spain. Plasma leptin concentrations were quantitatively determined in duplicates by the Luminex IS 100/200 system (Luminex Corp), with the xMAP technology by MILLIPLEX MAP, Human Angiogenesis/Growth Factor Magnetic Bead Panel 1 (EMD Millipore Corp). The intra-assay and inter-assay precision coefficients of variation for leptin were 2.8% and 6.0%, and sensitivity was 42.8 pg/mL. Plasma adiponectin concentrations were quantitatively determined in duplicates by the Luminex IS 100/200 system (Luminex Corp), with the FLEXMAP 3D technology by MILLIPLEX MAP, Human Adipokine Magnetic Bead Panel 1 (EMD Millipore Corp). For adiponectin, the intra- and inter-assay precision coefficients of variation were < 10% and < 15%, and sensitivity was 11 pg/mL.

2.4 | Bone measurements

Bone mass, fat mass and lean mass [body mass - (fat mass + bone mass)] were measured by dual-energy X-ray absorptiometry using the Hologic Discovery Wi (Hologic Inc., Bedford,). The equipment was calibrated at the start of each testing day by using a lumbar spine phantom as recommended by the manufacturer. APEX software, version 4.0.2 (Hologic Inc., Bedford,) was used to analyse the scans following the recommendations for children and adolescents.[21] The total body scan was used to obtain fat mass, lean mass, bone mineral content and areal bone mineral density at the total body less head and lumbar spine.

2.5 | Muscular fitness

Muscular fitness was assessed in laboratory conditions. We determined each participant's one repetition maximum when the child was able to lift throughout the full range of motion in bench press and leg press tests.[22] Participants received familiarisation sessions before the testing session to ensure an adequate technique, that is, controlled movements and proper breathing. Before attempting repetition maximum, participants performed six repetitions with a 50% estimated repetition maximum and three repetitions with a 90% estimated repetition maximum. Then, a series of single repetitions with increasing loads ranging from 0.5 to 2.3 kg for bench press and 10 to 20 kg for leg press were performed. The repetition maximum was determined when participants fell short of the full range of motion on at least two non-consecutive attempts. A resting time of 3–5 min between attempts was allowed.

2.6 | Statistical methods

Data were analysed using SPSS statistics, version 20 (IBM Corp) and statistical significance was defined as $p < 0.05$. Descriptive characteristics of participants are presented as mean ± standard deviation for continuous variables and percentages for categorical variables. All variables were checked for normality using visual check of histograms, Q-Q and box plots. Skewed data were log-transformed for analytical purposes. Sex interaction was checked in the association of adipokines with bone parameters. No significant interactions were found (all $p > 0.2$), so analyses were performed for boys and girls together.

Multiple regression analyses were carried out to examine the association between adipokines and bone parameters. Sex, years from peak height velocity and lean mass were introduced as covariates based on their known association with bone parameters.[23] The standardised regression coefficients (β) are reported, and the squared semi-partial correlation coefficients (sr^2) were used to determine the contribution of each predictor in the overall variance of the model after removing shared contributions with other predictors. Collinearity was checked for the variables using the variance inflation factor and tolerance levels.

Multiple linear regression analysis with interaction effect was used to test the role of muscular fitness in the association between adipokines (those that were significant in the multiple regression models) and bone parameters. The interaction effects of muscular fitness in the association between adipokines and bone parameters were further examined (in those with $p < 0.1$), stratifying by high/low (above/below study-specific median) levels of maximum repetition at bench press and leg press tests. Finally, moderation analyses were conducted using the PROCESS macro created by Hayes[24] to provide greater resolution for clarifying interactions. PROCESS uses ordinary least squares regression analysis when predicting continuous variables and a bias-corrected bootstrap method to estimate the conditional effects. In this regard, the Johnson–Neyman technique was used to test for significance along a continuous moderator variable and delineates the slope of the relationship at each value. In the context of the current study, the technique seeks for specific muscular fitness cut points in which the significant relationship between adipokines and bone parameters disappears. The moderation analyses were adjusted for sex, years from peak height velocity and lean mass.

3 | RESULTS

The descriptive characteristics of the children participating in the study are shown in Table 1. The mean age of the 84 participants (63% boys) was 10.0 ± 1.2 years and they were 2.3 ± 1.0 years below peak height velocity. The 26.2% of children were affected with overweight and 73.8% by obesity.

The association between adipokines and bone parameters is shown in Table 2. Leptin levels were negatively associated with lumbar spine bone mineral content after adjusting for sex, years from peak height velocity and lean mass ($\beta = -0.162$, $sr^2 = 0.022$, $p = 0.053$), whereas adiponectin was not associated with bone parameters. The interaction effects of muscular fitness in the association of leptin and lumbar spine bone mineral content are shown in

TABLE 1 Descriptive characteristics of the sample (mean ± standard deviation)

	All ($n = 84$)	Boys ($n = 53$)	Girls ($n = 31$)
Age (years)	10.0 ± 1.2	10.2 ± 1.2	9.7 ± 1.1
Years from PHV (years)	−2.3 ± 1.0	−2.7 ± 0.9	−1.8 ± 1.0
Height (cm)	143.8 ± 8.6	144.2 ± 7.9	142.9 ± 9.8
Body mass (kg)	55.3 ± 10.9	55.9 ± 10.5	54.2 ± 11.6
Body mass index (kg/m^2)	26.5 ± 3.5	26.7 ± 3.4	26.3 ± 3.6
Overweight (%)	26.2	24.5	29.0
Obesity (%)	73.8	75.5	71.0
Body composition			
Lean mass (kg)[a]	26.6 ± 5.0	27.2 ± 4.9	25.7 ± 5.2
Fat mass (kg)	22.3 ± 6.2	22.2 ± 5.9	22.4 ± 6.8
Adipokines			
Leptin (ng/mL)[a]	11.1 ± 5.8	11.4 ± 5.8	10.6 ± 5.8
Adiponectin (μg/mL)[a]	7.1 ± 5.3	7.8 ± 5.9	6.0 ± 3.9
Bone parameters			
TBLH BMC (g)[a]	961.11 ± 200.64	976.61 ± 206.05	934.60 ± 191.41
TBLH aBMD (g/cm^2)[a]	0.769 ± 0.058	0.774 ± 0.059	0.760 ± 0.056
LS BMC (g)[a]	24.86 ± 6.16	24.22 ± 6.17	25.94 ± 6.08
LS aBMD (g/cm^2)[a]	0.756 ± 0.082	0.737 ± 0.069	0.787 ± 0.093
Muscular fitness			
RM bench press (kg)	21.4 ± 4.4	22.4 ± 4.5	19.7 ± 3.8
RM leg press (kg)	134.9 ± 26.2	136.7 ± 28.2	132.0 ± 22.5

Abbreviation: *aBMD*, bone mineral density; *BMC*, bone mineral content; *LS*, lumbar spine; *PHV*, peak height velocity; *RM*, repetition maximum; *TBLH*, total body less head.

[a]Variables were log transformed for analytical purposes, but non-transformed variables are presented.

TABLE 2 Multiple regression models to examine the association between adipokines and bone parameters in children with overweight or obesity

Outcome	Predictors	β	sr^2	*p-value*	Outcome	Predictors	β	sr^2	*p-value*
TBLH BMC	Sex	−0.157	0.012	**0.009**	LS BMC	Sex	0.032	0.001	0.768
R^2 *adj* = 0.86	Years from PHV	0.390	0.041	**<0.001**	R^2 *adj* = 0.52	Years from PHV	0.407	0.045	**0.007**
	Lean mass	0.633	0.114	**<0.001**		Lean mass	0.431	0.053	**0.003**
	Leptin	−0.033	0.001	0.455		Leptin	−0.162	0.022	**0.053**
TBLH BMC	Sex	−0.137	0.001	**0.029**	LS BMC	Sex	0.037	0.001	0.743
R^2 *adj* = 0.85	Years from PHV	0.387	0.041	**<0.001**	R^2 *adj* = 0.49	Years from PHV	0.444	0.054	**0.004**
	Lean mass	0.622	0.125	**<0.001**		Lean mass	0.343	0.038	**0.014**
	Adiponectin	0.023	0.001	0.599		Adiponectin	0.048	0.002	0.553
TBLH aBMD	Sex	−0.107	0.005	0.279	LS aBMD	Sex	0.243	0.028	0.065
R^2 *adj* = 0.61	Years from PHV	0.271	0.019	**0.042**	R^2 *adj* = 0.32	Years from PHV	0.282	0.022	0.107
	Lean mass	0.597	0.102	**<0.001**		Lean mass	0.297	0.025	0.082
	Leptin	−0.059	0.003	0.433		Leptin	−0.088	0.007	0.370
TBLH aBMD	Sex	−0.118	0.007	0.234	LS aBMD	Sex	0.227	0.025	0.082
R^2 *adj* = 0.61	Years from PHV	0.286	0.023	**0.031**	R^2 *adj* = 0.32	Years from PHV	0.332	0.030	0.056
	Lean mass	0.561	0.102	**<0.001**		Lean mass	0.248	0.019	0.120
	Adiponectin	−0.016	0.000	0.825		Adiponectin	0.101	0.009	0.277

Bold type indicates $p \leq 0.05$.

β is the estimated standardised regression coefficient.

sr^2 = Semi-partial correlation coefficients reflecting adipokines explanatory value after accounting for the other variables included in the model.

Abbreviations: *aBMD*, bone mineral density; *BMC*, bone mineral content; *LS*, lumbar spine; *PHV*, peak height velocity; *TBLH*, total body less head.

TABLE 3 Multiple linear regression analyses with interaction effect for testing the role of muscular fitness in the association between leptin and bone parameters in children with overweight or obesity

Outcome	Predictors	β	*p-value*	Outcome	Predictors	β	*p-value*
LS BMC	Sex	0.068	0.537	LS BMC	Sex	0.042	0.704
R^2 *adj* = 0.53	Years from PHV	0.428	**0.004**	R^2 *adj* = 0.53	Years from PHV	0.428	**0.005**
	Lean mass	0.368	**0.012**		Lean mass	0.469	**0.004**
	Leptin	−0.349	0.338		Leptin	−0.767	**0.029**
	RM bench press	−0.036	0.909		RM leg press	−0.552	0.062
	Leptin x RM bench press	0.284	0.543		Leptin x RM leg press	0.887	0.074 [a]

Bold type indicates $p \leq 0.05$.

β is the estimated standardised regression coefficient.

RM bench press determined the muscular fitness at the upper body.

RM leg press determined the muscular fitness at the lower body.

Abbreviations: *BMC*, bone mineral content; *LS*, lumbar spine; *PHV*, peak height velocity; *RM*, repetition maximum.

[a] *p interaction <0.1*.

Table 3. After adjusting for the same set of covariates, no significant interaction was found for either muscular fitness at the upper body ($p = 0.543$) or muscular fitness at the lower body ($p = 0.074$) in the association of leptin with lumbar spine bone mineral content.

Figure 1 depicts the regression slopes of leptin with lumbar spine bone mineral content, as a function of muscular fitness at the lower body. The results revealed a significant inverse association between leptin and lumbar spine bone mineral content in the low maximum repetition group ($\beta = -0.314$, $p = 0.022$), whereas no evidence of association was found in the high maximum repetition group ($\beta = -0.046$, $p = 0.693$). Figure 2 shows the regression slope estimates and the 95% confidence intervals for the association between leptin and lumbar spine bone mineral content as a function of maximum repetition at leg press test. The Johnson–Neyman technique revealed that the significant inverse association between leptin and lumbar spine bone mineral content became non-significant when repetition maximum at leg press test was above 133.3 kg. Of note, 51.2% of sample were above of this cut-off point.

4 | DISCUSSION

In the present study, we observed that leptin was negatively associated with lumbar spine bone mineral content in children with overweight or obesity. In addition, there was evidence for an interaction between leptin and muscular fitness at the lower body on lumbar spine bone mineral content. Indeed, higher leptin levels were related to lower lumbar spine bone mineral content in those children with overweight or obesity that had low maximum repetition results at leg press test. Likewise, those children whose repetition maximum at leg press test was above 133.3 kg could overcome the negative influence of leptin on lumbar spine bone mineral content. To our knowledge, this is one of the few studies that thoroughly addresses the association between adipokines and bone parameters in children with overweight or obesity, and the first study examining the role of muscular fitness in this relationship.

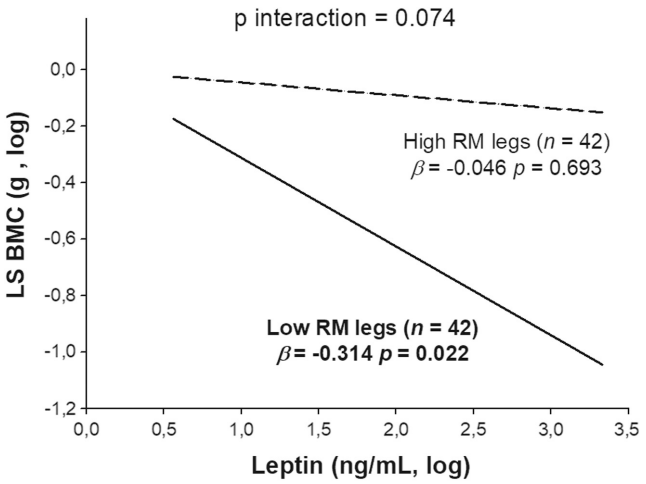

FIGURE 1 Graphical representation of the regression slopes between leptin and lumbar spine bone mineral content by levels of muscular fitness at the lower body. Low and high levels were defined as being below/above the study-specific median value for RM leg press. The regression models were adjusted for sex, years from peak height velocity and lean mass. *BMC* bone mineral content; *LS* lumbar spine; *RM* repetition maximum

Our results showed a negative association between leptin and lumbar spine bone mineral content after adjusting for sex, years from peak height velocity and lean mass. This finding agreed with a longitudinal study where leptin levels at 12 years of age were inversely associated with bone mass increments at the total body, femoral neck and lumbar spine regions over the next 24 months in boys.[25] Similar to these results, Meng et al.[7] implemented a two-sample mendelian randomisation and found a negative association between leptin and lumbar spine areal bone mineral density. In the same way, Tamme et al.[26] found that leptin–adiponectin ratio, which is directly proportional to leptin levels and inversely proportional to adiponectin levels, was negatively correlated with lumbar spine areal bone mineral density and lumbar spine bone mineral apparent density in 18-year-old boys after controlling for fat mass percentage, testosterone, physical activity and homeostatic model assessment for insulin resistance. However, we found no association between leptin–adiponectin ratio and bone parameters (data not shown). Given that low levels of leptin increase trabecular bone volume in mice,[27] it is reasonable that leptin may affect the spine because of the greater trabecular bone proportion.[7]

A previous observational study found a positive correlation between leptin levels and total body areal bone mineral density in obese prepubertal, early pubertal and late pubertal children.[10] These opposite results could be explained by the fact that Vishnevskaya and Solntsava did not control by potential cofounders. Nevertheless,

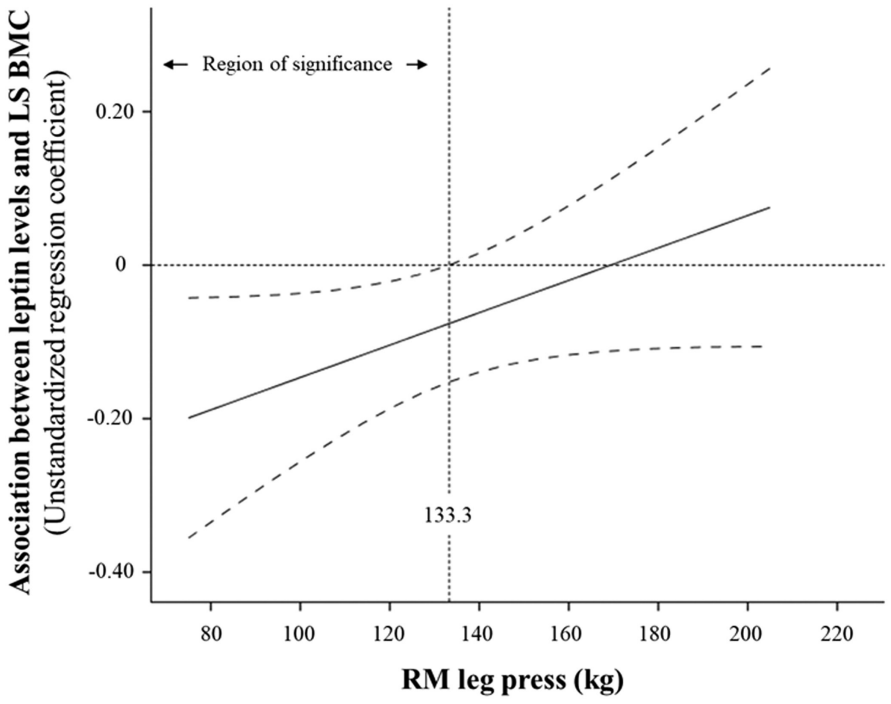

FIGURE 2 Regression slope estimate and 95% confidence interval for the association between leptin and lumbar spine bone mineral content as a function of muscular fitness at the lower body, based on Johnson–Neyman results. The analyses were adjusted for sex, years from peak height velocity and lean mass. RM leg press determined the muscular fitness at the lower body. *BMC* bone mineral content; *LS* lumbar spine; *RM* repetition maximum

other studies did not find any association between leptin and bone parameters in adolescents.[11,12,28] Altogether, these controversial results may be explained by the dual pathway through which leptin levels affects bone metabolism. In the central pathway, leptin may suppress osteoblast proliferation and promotes the resorption of the osteoclasts. Otherwise, in the peripheral pathway, leptin may enhance the proliferation and differentiation of bone marrow mesenchymal stem cells into osteoblastic lineage.[7]

A few studies have examined the association between adiponectin and bone parameters in children with overweight or obesity. The results of the present investigation showed no significant association between adiponectin and bone parameters after adjusting for sex, years from peak height velocity and lean mass. Corroborating with our findings, two studies have reported no association between adiponectin levels and bone parameters in girls[11] and boys.[12] Nevertheless, Sayers et al.[13] found a negative association of adiponectin levels with bone parameters in boys and girls even after controlling for lean mass, fat mass and height. Together with the abovementioned relationship between leptin–adiponectin ratio and lumbar spine bone parameters, it seems that higher adiponectin levels might have a negative effect on bone mass in youth. Overall, the conflicting results may be explained by the moderated effect of sex hormones in the relationship between adiponectin and bone parameters since a negative correlation was seen in females but not in males.[9]

Importantly, we also considered the interaction effect of muscular fitness. Despite no significant result was found, there was evidence for an interaction between leptin and muscular fitness at the lower body on lumbar spine bone mineral content and therefore, we explored this association stratifying by high/low levels of muscular fitness at the lower body. This trended interaction might become significant if a bigger sample size was available. Then, we found that high maximum repetition results at leg press test eliminated the detrimental effect of leptin on lumbar spine bone mineral content. Moreover, the role of maximum repetition at leg press test was evident from 133.3 kg. This result is in line with previous studies that found a negative association between muscular fitness and leptin levels in children.[16,17] In this regard, heavier individuals have higher levels of muscular fitness because they carry more body mass and therefore, higher muscular contractions are needed. According to our results, the minimum effective strain magnitude to enhance bone adaptation may be guaranteed when a high muscular fitness is acquired in children with overweight or obesity. In addition, we ran the analyses using field-based muscular fitness tests and we found similar results with the absolute handgrip strength and standing long jump (Table S1, Figure S1 and Figure S2), which reinforces the findings of the present study.

5 | STRENGTHS AND LIMITATIONS

We acknowledged several limitations of our study. First, our findings are limited due to its cross-sectional design and causal direction cannot be inferred. Second, unconsidered confounding variables may also affect the findings based on observational data. Third, the number of participants with complete data in all studied variables is relatively small. Fourth, we used plasma samples to measure adipokines while previous studies have used plasma or serum samples and therefore, comparisons should be considered with caution. However, as shown in a study the correlations between plasma and serum measurements suggest that the differences in metabolite concentrations does not necessarily introduce a bias in cross-sectional studies.[29] Fifth, the lumbar spine bone parameters were obtained from the whole-body scan and thus, caution should be used when comparing with lumbar spine bone parameters obtained from the regional scan. Nevertheless, children with overweight or obesity tend to have high levels of lumbar spine bone parameters and this fact reduces the difference between the whole-body and the regional scans.[30] Sixth, peripheral quantitative computed tomography parameters were not assessed and therefore, we could not know if adipokines affects to the cortical or trabecular compartments. On the other hand, the use of DXA and the accuracy of the objective methodology used for muscular fitness and blood measurements are strengths of this study.

6 | CONCLUSION

This study supported the fact that bone mass is regulated by a central pathway that is dependent of leptin concentrations. It showed that higher leptin concentrations were associated with lower lumbar spine bone mineral content in children with overweight or obesity. Furthermore, our data suggest that higher levels of muscular fitness at the lower body may ameliorate or even fully eliminate this association. These results reinforce the importance of achieving proper muscular fitness levels at the lower body to preserve normal bone accrual in this population. Future longitudinal and intervention studies are needed to confirm these findings.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

José J. Gil-Cosano https://orcid.org/0000-0003-0723-876X
Luis Gracia-Marco https://orcid.org/0000-0002-4020-0256
Esther Ubago-Guisado https://orcid.org/0000-0002-9397-2399
Jairo H. Migueles https://orcid.org/0000-0003-0366-6935
Daniel Courteix https://orcid.org/0000-0001-6918-714X
Idoia Labayen https://orcid.org/0000-0002-4334-3287
Abel Plaza-Florido https://orcid.org/0000-0002-5374-3129
Pablo Molina-García https://orcid.org/0000-0001-6888-0997
Frédéric Dutheil https://orcid.org/0000-0002-1468-6029
Francisco B. Ortega https://orcid.org/0000-0003-2001-1121

REFERENCES

1. Rizzoli R, Bianchi ML, Garabédian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone. 2010;46(2):294-305. doi:10.1016/j.bone.2009.10.005

2. van Leeuwen J, Koes BW, Paulis WD, van Middelkoop M. Differences in bone mineral density between normal-weight children and children with overweight and obesity: a systematic review and meta-analysis. Obes Rev. 2017;18(5):526-546. doi:10.1111/obr.12515
3. Sioen I, Lust E, de Henauw S, Moreno LA, Jiménez-Pavón D. Associations between body composition and bone health in children and adolescents: a systematic review. Calcif Tissue Int. 2016;99(6):557-577. doi:10.1007/s00223-016-0183-x
4. Dimitri P. The impact of childhood obesity on skeletal health and development. J Obes Metab Syndr. 2019;28(1):4-17. doi:10.7570/JOMES.2019.28.1.4
5. Fintini D, Cianfarani S, Cofini M, et al. The bones of children with obesity. Front Endocrinol. 2020;11(April):1-16. doi:10.3389/fendo.2020.00200
6. Münzberg H, Morrison CD. Structure, production and signaling of leptin. Metabolism. 2015;64(1):13-23. doi:10.1016/j.metabol.2014.09.010
7. Meng XH, Tan LJ, Xiao HM, Tang BS, Deng HW. Examining the causal role of leptin in bone mineral density: a mendelian randomization study. Bone. 2019;125:25-29. doi:10.1016/j.bone.2019.05.006
8. Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin - a key adipokine in the metabolic syndrome. Diabetes Obes Metab. 2006;8(3):264-280. doi:10.1111/j.1463-1326.2005.00510.x
9. Bi X, Loo YT, Henry CJ. Relationships between adiponectin and bone: sex difference. Nutrition. 2020;70:110489. doi:10.1016/j.nut.2019.04.004
10. Vishnevskaya M, Solntsava A. Bone mineral density in obese children. Pediatr Res. 2011;70:402.
11. Huang KC, Cheng WC, Yen RF, Tsai KS, Tai TY, Yang WS. Lack of independent relationship between plasma adiponectin, leptin levels and bone density in nondiabetic female adolescents. Clin Endocrinol (Oxf). 2004;61(2):204-208. doi:10.1111/j.1365-2265.2004.02081.x
12. Vaitkeviciute D, Lätt E, Mäestu J, et al. Adipocytokines and bone metabolism markers in relation to bone mineral values in early pubertal boys with different physical activity. J Pediatr Endocrinol Metabolism. 2016;29(6):723-729. doi:10.1515/jpem-2015-0282
13. Sayers A, Timpson NJ, Sattar N, et al. Adiponectin and its association with bone mass accrual in childhood. J Bone Miner Res. 2010;25(10):2212-2220. doi:10.1002/jbmr.116
14. García-Hermoso A, Ramírez-Campillo R, Izquierdo M. Is muscular fitness associated with future health benefits in children and adolescents? A systematic review and meta-analysis of longitudinal studies. Sports Med. 2019;49(7):1079-1094. doi:10.1007/s40279-019-01098-6
15. Ubago-Guisado E, Vlachopoulos D, Ferreira de Moraes AC, et al. Lean mass explains the association between muscular fitness and bone outcomes in 13-year-old boys. Acta Paediatr. 2017;106(10):1658-1665. doi:10.1111/apa.13972
16. Delgado-Alfonso A, Pérez-Bey A, Conde-Caveda J, et al. Independent and combined associations of physical fitness components with inflammatory biomarkers in children and adolescents. Pediatr Res. 2018;84(5):704-712. doi:10.1038/s41390-018-0150-5
17. Steene-Johannessen J, Kolle E, Andersen LB, Anderssen SA. Adiposity, aerobic fitness, muscle fitness, and markers of inflammation in children. Med Sci Sports Exerc. 2013;45(4):714-721. doi:10.1249/MSS.0b013e318279707a
18. Cadenas-Sánchez C, Mora-González J, Migueles JH, et al. An exercise-based randomized controlled trial on brain, cognition, physical health and mental health in overweight/obese children (ActiveBrains project): rationale, design and methods. Contemp Clin Trials. 2016;47:315-324. doi:10.1016/j.cct.2016.02.007
19. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes. 2012;7(4):284-294. doi:10.1111/j.2047-6310.2012.00064.x
20. Moore SA, McKay HA, Macdonald H, et al. Enhancing a somatic maturity prediction model. Med Sci Sports Exerc. 2015;47(8):1755-1764. doi:10.1249/MSS.0000000000000588
21. Crabtree NJ, Arabi A, Bachrach LK, et al. Dual-energy x-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD pediatric official positions. J Clin Densitom. 2014;17(2):225-242. doi:10.1016/j.jocd.2014.01.003
22. Faigenbaum AD, Milliken LA, Westcott WL. Maximal strength testing in healthy children. J Strength Cond Res. 2003;17(1):162-166. doi:10.1519/1533-4287(2003)017<0162
23. Ubago-Guisado E, Vlachopoulos D, Fatouros IG, et al. Longitudinal determinants of 12-month changes on bone health in adolescent male athletes. Arch Osteoporosis. 2018;13(1):106. doi:10.1007/s11657-018-0519-4
24. Hayes A. Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach. (1st ed). Guilford Press; 2013.
25. Vaitkeviciute D, Lätt E, Mäestu J, et al. Longitudinal associations between bone and adipose tissue biochemical markers with bone mineralization in boys during puberty. BMC Pediatr. 2016;16(1):1-7. doi:10.1186/s12887-016-0647-1
26. Tamme R, Jürimäe J, Mäestu E, et al. Leptin to adiponectin ratio in puberty is associated with bone mineral density in 18-year-old males. Bone Rep. 2021;2022(16):6-10. doi:10.1016/j.bonr.2021.101158
27. Ducy P, Amling M, Takeda S, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell. 2000;100(2):197-207. doi:10.1016/S0092-8674(00)81558-5
28. Roemmich JN, Clark PA, Mantzoros CS, Gurgol CM, Weltman A, Rogol AD. Relationship of leptin to bone mineralization in children and adolescents. J Clin Endocrinol Metabol. 2003;88(2):599-604. doi:10.1210/jc.2001-012025
29. Yu Z, Kastenmüller G, He Y, et al. Differences between human plasma and serum metabolite profiles. PLoS ONE. 2011;6(7):e21230. doi:10.1371/journal.pone.0021230
30. Nysom K, Mølgaard C, Michaelsen KF. Bone mineral density in the lumbar spine as determined by dual-energy x-ray absorptiometry: comparison of whole-body scans and dedicated regional scans. Acta Radiol. 1998;39(6):632-636. doi:10.3109/02841859809175488

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Gil-Cosano JJ, Gracia-Marco L, Ubago-Guisado E, Migueles JH, Courteix D, Labayen I, Leptin levels were negatively associated with lumbar spine bone mineral content in children with overweight or obesity. Acta Paediatr. 2022;111:1966–1973. https://doi.org/10.1111/apa.16456