Pediatric RESEARCH

BASIC SCIENCE ARTICLE

Inflammatory markers and bone mass in children with overweight/obesity: the role of muscular fitness

This article has been corrected since Advance Online Publication and a correction is also printed in this issue

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OBJECTIVES: To examine which inflammatory markers are associated with bone mass and whether this association varies according to muscular fitness in children with overweight/obesity.
METHODS: Plasma interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), epidermal growth factor, vascular endothelial growth factor A (VEGF), and C-reactive protein were analyzed in 55 children aged 8–11 years. A muscular fitness score was computed. Bone mineral content (BMC) of the total body-less head (TBLH) and lumbar spine (LS) were assessed using dual-energy x-ray absorptiometry.
RESULTS: IL-6 ($\beta = -0.136$) and VEGF ($\beta = -0.099$) were associated with TBLH BMC, while TNF-α ($\beta = -0.345$) and IL-1β ($\beta = 0.212$) were associated with LS BMC ($P < 0.05$). The interaction effect of muscular fitness showed a trend in the association of VEGF with TBLH BMC ($P = 0.122$) and TNF-α with LS BMC ($P = 0.057$). Stratified analyses by muscular fitness levels showed an inverse association of VEGF with TBLH BMC ($\beta = -0.152$) and TNF-α with LS BMC ($\beta = -0.491$) in the low-fitness group, while no association was found in the high-fitness group.
CONCLUSION: IL-6, VEGF, TNF-α, and IL-1β are significantly associated with bone mass. Higher muscular fitness may attenuate the adverse effect of high VEGF and TNF-α on bone mass.

Pediatric Research (2020) 87:42–47; https://doi.org/10.1038/s41390-019-0572-8

INTRODUCTION

Obesity and osteoporosis are two major global health problems with an increasing prevalence and closely related to both mortality and morbidity worldwide.[1,2] The belief that obesity is protective against osteoporosis has recently come into question due to the increasing evidence about the endocrine function and interplay between different tissues, such as muscular, adipose, and bone tissue.[3] Moreover, childhood is a critical period for bone accretion[4] and reaching an optimal peak bone mass is considered the best protective factor against future osteoporosis and fracture.

The link between body composition and bone health in children and adolescents has been the focus of various investigations over the past few decades.[5] Previous cross-sectional evidence highlights a negative association between fat mass (FM) and bone mass in adolescents, once lean mass (LM) is accounted for.[6] In addition, Mengel et al.[7] observed that boys with overweight/obesity who had an extensive body mass index (BMI) gain during puberty experienced lower gains in bone outcomes. In this regard, the increasing presence of fat within the bone marrow is known to affect osteoblast differentiation, increasing osteoclastic activity and affecting mineralization.[8]

The pediatric skeleton is sensitive to factors that influence bone accrual, including physical activity and increased inflammatory cytokines.[9] In this sense, the role that inflammatory markers play in the child's skeleton has been investigated.[10–12] For instance, Mengel et al.[11] found that vascular endothelial growth factor A (VEGF) was inversely associated with total body bone mineral content (BMC), whereas epidermal growth factor (EGF) was inversely associated with areal bone mineral density (aBMD) and apparent BMD at the lumbar spine (LS). Similar to adipose tissue, the skeletal muscle is a secretory organ responsible for the production of several hundreds of myokines in response to exercise.[13] Thus, the muscle–adipose tissue axis should be taken into consideration to elucidate the systemic effects of the inflammatory markers on bone health.

Muscular fitness has been favorably associated with potential health benefits (i.e., bone health, mental health, total and central adiposity, cardiovascular disease, and metabolic risk factors) in

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Received: 13 June 2019 Revised: 2 August 2019 Accepted: 16 August 2019
Published online: 7 September 2019

children and adolescents.[14] Recent studies reported that the association between muscular fitness and bone outcomes was explained by LM in different growth stages.[15–17] In addition, muscular fitness has been inversely associated with C-reactive protein (CRP) in adolescents with overweight/obesity[18] and also in prepubertal children.[19] In adolescents, a clustered score of inflammatory markers, including CRP, C3, C4, fibrinogen, and leptin, has been inversely associated with muscular fitness.[20]

To the best of our knowledge, no study has tested the role of muscular fitness in the association between inflammatory markers and bone mass. Therefore, the purpose of this study was twofold: (1) to identify which inflammatory markers are associated with bone mass in children with overweight/obesity and (2) to examine whether this association varies according to muscular fitness levels in this population.

MATERIAL AND METHODS

Participants and study design

The present cross-sectional study was developed within the ActiveBrains project framework (ClinicalTrial.gov ID: NCT02295072). A detailed description of the study design, purpose, methodology, and inclusion/exclusion criteria has been published elsewhere.[21] The ActiveBrains project measured 110 prepubertal children with overweight/obesity from Granada (Southern Spain). Participants were recruited from the Pediatric Unit of the University Hospitals San Cecilio and Virgen de las Nieves. The study protocol was approved by the Review Committee for Research Involving Human Subjects at the University of Granada (Reference: 848, February 2014) and informed consent was obtained from parents.

In this report, a total of 55 children (10.2 ± 1.2 years, 38 boys) with complete data on inflammatory markers, body composition (i.e., bone, FM, and LM), objectively measured muscular fitness, and sexual maturation assessment were included (see flowchart in Fig. 1).

Anthropometry and sexual maturation

Body mass (kg) was measured with an electronic scale (SECA 861, Hamburg, Germany). Height (cm) and sitting height were measured with a precision stadiometer (SECA 225, Hamburg, Germany). BMI was calculated as: body mass (kg)/height (m^2) and the participants were classified into BMI categories according to the World Obesity Federation criteria.[22]

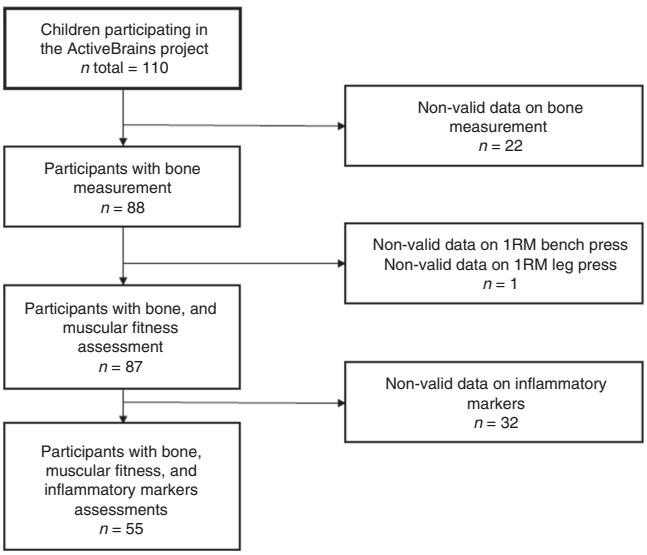

Fig. 1 Flowchart of study participants. DXA, dual-energy x-ray absorptiometry; 1RM, one repetition maximum

Somatic maturity offset was assessed as years from peak height velocity (PHV) from age, height, and sitting height using validated algorithms for children.[23]

Inflammatory markers

Venous blood samples were obtained between 8:00 a.m. and 9:00 a.m. by venipuncture after an overnight fast (at least 12 h) in all participants. Blood samples in tubes containing EDTA were spun immediately at 1000 × *g* for 10 min. Plasma was isolated and stored at −80 °C until analyses. Three key cytokines analyzed in the plasma were included in this study: interleukin-6 (IL-6, pg/mL), IL-1β (pg/mL), and tumor necrosis factor-α (TNF-α, pg/mL). IL-6, IL-1β, and TNF-α were quantified by multiple analyte profiling technology (MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel, EMD Millipore Corporation, Missouri, USA) with a kit plex (HCYIL6-MAG Anti-Human IL-6 Beads set, HCYIL1B-MAG Anti-Human IL-1β Bead, and HCYTNFA-MAG Anti-Human TNF-α Beads set), using one 96-well plate with sealers (Cat. HSTCMAG-28SK). The intra- and inter-assay precision coefficients of variation for IL-6 were 5% and 20%, respectively, and sensitivity was 0.11 pg/mL. For both IL-1β and TNF-α, the intra- and inter-assay precision coefficients of variation were 5% and 15%, respectively, with a sensitivity of 0.14 pg/mL for IL-1β and of 0.16 pg/mL for TNF-α. CRP (mg/L) was determined by immunoturbidimetry (Alinity c CRP Vario Reagent Kit ref. 07P5620, Abbott, Illinois, USA) with a sensitivity of 0.40 mg/L and an intra-assay coefficient of variation of 1.1%.

Two growth factors were analyzed by multiple analyte profiling technology (MILLIPLEX® MAP Human Angiogenesis/Growth Factor Magnetic Bead Panel 1, EMD Millipore Corporation, Missouri, USA) with a kit plex (HVEGF-MAG Anti-Human VEGF A Bead and HAGEGF-MAG Anti-Human EGF Bead), using one 96-well plate with sealers (Cat. HAGP1MAG-12K). The intra- and inter-assay precision coefficients of variation for VEGF A (pg/mL) were 3.5% and 10%, respectively, and sensitivity was 8.1 pg/mL. For EGF (pg/mL), the intra- and inter-assay precision coefficients of variation were 3.2% and 6.8%, respectively, with a sensitivity of 1.0 pg/mL.

Body composition

Children were scanned with dual-energy x-ray absorptiometry (DXA) using the Hologic Discovery Wi (Hologic Series Discovery QDR, Bedford, MA, USA). The DXA equipment was calibrated at the start of each testing day by using a LS phantom as recommended by the manufacturer. All DXA scans and analyses were performed using the GE encore software (version 4.0.2) and were completed following the same protocol by the same researcher. The positioning of the participants and the analyses of the results were undertaken following recommendations from the International Society of Clinical Densitometry.[24] The total body scan was used to obtain FM, LM, and BMC at the total body-less head (TBLH) and at the LS.

Objectively measured muscular fitness

Muscular fitness was evaluated in laboratory conditions. We determined each participant's 1 repetition maximum (1RM) when the child was able to lift throughout the full range of motion in bench press and leg press tests.[25] Participants received familiarization sessions before the testing session in order to ensure an adequate technique (i.e., controlled movements and proper breathing). Before attempting 1RM, participants performed six repetitions with a light load and three repetitions with a heavier load (50–90% estimated 1RM). Then, a series of single repetitions with increasing loads (0.5–2.3 kg for bench press and 10–20 kg for leg press) were performed. The 1RM 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. Rate of perceived exertion at each attempt was obtained using the children's OMNI-Resistance Exercise scale.[26] Moreover, during all testing procedures researchers obtained more information from the participants by asking questions such as: "How do you feel?," "Is the

load light, medium or heavy?," and "Could you lift more?" to aid in the progression of the 1RM trials.

A muscular fitness score was computed by combining the standardized values of 1RM bench press and 1RM leg press tests. Each of these variables was standardized as follows: standardized value = (value − mean)/SD. The muscular fitness *z*-score was calculated as the mean of the two standardized scores (1RM bench press and 1RM leg press).

Statistical analyses

Data were analyzed using SPSS IBM statistics (version 20 for Windows, Chicago, IL) and the normal distribution of the raw variables was confirmed using visual check of histograms, Q–Q, and box plots. Statistical significance was defined as $P < 0.05$. Interaction analyses were performed between sex and inflammatory markers on the outcomes. No significant interactions were found ($P > 0.05$), so analyses were carried out for boys and girls together.

Descriptive characteristics of participants are presented as mean ± standard deviation (SD). Differences between sexes were determined by independent *t* tests. Stepwise hierarchical regression analyses were carried out to identify the inflammatory markers that best predicted bone mass. Sex, years from PHV, and TBLH LM were considered for entry into step 1 of the model, and subsequent addition of inflammatory markers in step 2 was conducted to determine the contribution to the bone mass variables following step 1 adjustments. These covariates were selected because of their known association with bone mass.[27] The standardized 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.

Finally, multiple linear regression analysis with interaction effect was used to test the role of muscular fitness in the association between inflammatory markers (those that were significant in the stepwise regression models) and bone mass. The interaction effects of muscular fitness in the association between inflammatory markers and bone mass were further examined (in those with $P < 0.20$), stratifying by high/low (above/below sex-, age-, and study-specific median) levels of muscular fitness.

RESULTS

Descriptive characteristics are presented in Table 1 (mean ± SD). Girls were more mature than boys ($P < 0.05$), but no significant differences between sexes were found in the remaining descriptive variables. The mean age of the participants was 10 ± 1.2 years and they were 2.3 ± 1.0 years below PHV, overweight, and obesity was evident in 30.9% and 69.1% of them, respectively.

Table 2 shows the stepwise multiple regression analyses for identifying the inflammatory markers that explained the variance in the outcome variables in children with overweight/obesity. The probability of F-to-remove ≥0.1 was established in order to identify these markers. For TBLH BMC, 88% of the variance was explained by TBLH LM, years from PHV, IL-6, sex, and VEGF ($sr^2 = 0.009–0.135$), while 66% of the variance in LS BMC was explained by TNF-α, years from PHV, TBLH LM, IL-1β, and sex ($sr^2 = 0.001–0.096$).

The role of muscular fitness *z*-score in the association of inflammatory markers (those previously included in the stepwise method) and bone mass is shown in Table 3. After adjusting for sex, years from PHV, and TBLH LM, the interaction effect of muscular fitness showed a positive trend in the association of VEGF with TBLH BMC ($P = 0.122$) and TNF-α with LS BMC ($P = 0.057$). No evidence of interaction with muscular fitness was found in the remaining associations of IL-6 with TBLH BMC ($P = 0.857$) and IL-1β with LS BMC ($P = 0.309$).

Table 1. Descriptive characteristics of the study sample

	All ($n = 55$)	Boys ($n = 38$)	Girls ($n = 17$)
Age (years)	10.2 ± 1.2	10.3 ± 1.2	9.9 ± 1.2
Height (cm)	144.5 ± 8.8	144.9 ± 7.4	143.5 ± 11.5
Body mass (kg)	56.2 ± 11.3	56.9 ± 10.4	54.4 ± 13.3
BMI (kg/m^2)	26.7 ± 3.7	26.9 ± 3.7	26.0 ± 3.6
Overweight (%)	30.9	28.9	35.3
Obesity (%)	69.1	71.1	64.7
Years from PHV (years)	−2.3 ± 1.0	−2.6 ± 0.8	−1.6 ± 1.2
Inflammatory markers			
IL-1β (pg/mL)[a]	1.7 ± 0.9	1.6 ± 0.9	1.9 ± 0.7
IL-6 (pg/mL)[a]	1.8 ± 1.3	1.8 ± 1.4	1.8 ± 1.0
TNF-α (pg/mL)[a]	4.1 ± 1.6	4.1 ± 1.7	3.9 ± 1.3
EGF (pg/mL)[a]	9.3 ± 20.8	7.9 ± 18.0	12.3 ± 26.5
VEGF (pg/mL)[a]	55.1 ± 51.1	49.0 ± 41.7	68.5 ± 67.1
CRP (mg/L)[a]	3.3 ± 3.1	3.6 ± 3.5	2.4 ± 1.6
Body composition			
TBLH BMC (g)[a]	988.42 ± 197.87	998.50 ± 186.18	965.88 ± 226.29
LS BMC (g)[a]	25.01 ± 6.18	24.03 ± 5.75	27.22 ± 6.71
TBLH LM (kg)[a]	27.3 ± 5.2	27.7 ± 4.5	26.6 ± 6.5
TBLH FM (kg)[a]	22.4 ± 6.4	22.8 ± 6.3	21.6 ± 6.9
Objective muscular fitness			
1RM bench press (kg)	22.1 ± 4.4	22.8 ± 4.7	20.4 ± 3.2
1RM leg press (kg)	138.2 ± 26.4	139.1 ± 26.6	136.1 ± 26.4
Muscular fitness *z*-score[b]	0.0 ± 1.0	0.1 ± 1.1	−0.3 ± 0.8

Data are presented as mean ± standard deviation
BMI body mass index, *PHV* peak height velocity, *IL* interleukin, *TNF-α* tumor necrosis factor-α, *EGF* epidermal growth factor, *VEGF* vascular endothelial growth factor A, *CRP* c-reactive protein, *TBLH* total body-less head, *LS* lumbar spine, *BMC* bone mineral content, *LM* lean mass, *FM* fat mass, *1RM* one maximum repetition
[a]Values were Blom transformed before analysis, but non-transformed values are presented
[b]*Z*-score mean computed from 1RM bench press (kg) and 1RM leg press (kg) tests

Figure 2 shows the standardized β regression slopes of VEGF with TBLH BMC and TNF-α with LS BMC, according to muscular fitness levels. Stratified analyses by muscular fitness levels (below/above median) showed a significant inverse association between VEGF and TBLH BMC in the low muscular fitness group (Fig. 2a, $\beta = -0.152$, $P = 0.032$), while no evidence of association was found in the high muscular fitness group (Fig. 2a, $\beta = -0.045$, $P = 0.598$). Likewise, an inverse association between TNF-α and LS BMC was found in the low muscular fitness group (Fig. 2b, $\beta = -0.491$, $P < 0.001$), although this association was non-significant in the high muscular fitness group (Fig. 2b, $\beta = -0.060$, $P = 0.666$).

DISCUSSION

In the present study, we showed IL-6 and VEGF to be associated with TBLH BMC and TNF-α and IL-1β with LS BMC in children with overweight/obesity. In addition, our results suggested that higher levels of muscular fitness may attenuate the adverse effects of VEGF and TNF-α on TBLH BMC and LS BMC, respectively. To the best of our knowledge, this is one of the few studies that thoroughly addresses the influence of inflammatory markers on bone mass, and the first study examining the role of muscular fitness in the relationship between inflammatory markers and bone mass.

Inflammatory markers and bone mass in overweight/obese children

In this study, an inverse association between VEGF and TBLH BMC was found after controlling for the effect of sex, years from

Table 2. Stepwise hierarchical regression models to identify the inflammatory markers that best predict bone mass in children with overweight/obesity ($n = 55$)

Outcome	Predictors	β STD	sr^2	*P* value	Outcome	Predictors	β STD	sr^2	*P* value
TBLH BMC	Sex	−0.140	0.009	**0.038**	LS BMC	Sex	0.039	0.001	0.742
R^2 adj = 0.88	Years from PHV	0.376	0.039	**<0.001**	R^2 adj = 0.66	Years from PHV	0.427	0.049	**0.008**
	TBLH LM	0.637	0.135	**<0.001**		TBLH LM	0.342	0.039	**0.017**
	IL-6	−0.136	0.019	**0.006**		TNF-α	−0.345	0.096	**<0.001**
	VEGF	−0.099	0.009	**0.040**		IL-1β	0.212	0.035	**0.024**

IL-1β, IL-6, TNF-α, EGF, VEGF, and CRP were introduced in the step 2, but only those that were included by the stepwise method are shown. Boldface indicates $P < 0.050$

β STD estimated standardized regression coefficient of the focal fitness test, sr^2 semi-partial correlation coefficients reflecting inflammatory explanatory value after accounting for the other variables included in the model, *PHV* peak height velocity, *IL* interleukin, *TNF-α* tumor necrosis factor-*α*, *EGF* epidermal growth factor, *VEGF* vascular endothelial growth factor A, *CRP* C-reactive protein, *TBLH* total body-less head, *LS* lumbar spine, *BMC* bone mineral content, *LM* lean mass

Table 3. Multiple linear regression analyses with interaction effect for testing the role of muscular fitness in the association between inflammatory markers and bone mass variables in children with overweight/obesity ($n = 55$)

Outcome	Predictors	β STD	*P* value	Outcome	Predictors	β STD	*P* value
TBLH BMC	Sex	−0.135	0.057	LS BMC	Sex	0.079	0.557
R^2 adj = 0.87	Years from PHV	0.385	**<0.001**	R^2 adj = 0.55	Years from PHV	0.479	**0.009**
	TBLH LM	0.581	**<0.001**		TBLH LM	0.307	0.085
	VEGF	−0.073	0.179		IL-1β	0.080	0.404
	MF *z*-score[a]	0.072	0.259		MF *z*-score[a]	0.060	0.595
	VEGF × MF	0.081	0.122[b]		IL-1β × MF	0.102	0.309
TBLH BMC	Sex	−0.141	**0.049**	LS BMC	Sex	0.106	0.363
R^2 adj = 0.88	Years from PHV	0.382	**<0.001**	R^2 adj = 0.64	Years from PHV	0.385	**0.018**
	TBLH LM	0.580	**<0.001**		TBLH LM	0.330	**0.037**
	IL-6	−0.126	**0.017**		TNF-α	−0.205	**0.024**
	MF *z*-score[a]	0.098	**0.093**		MF *z*-score[a]	0.010	0.914
	IL-6 × MF	0.009	0.857		TNF-α × MF	0.175	0.057[b]

Boldface indicates $P < 0.050$

β STD estimated standardized regression coefficient of the focal fitness test, *PHV* peak height velocity, *VEGF* vascular endothelial growth factor A, *IL* interleukin, *TNF-α* tumor necrosis factor-*α*, *MF* muscular fitness, *TBLH* total body-less head, *BMC* bone mineral content, *LM* lean mass

[a]*Z*-score mean computed from 1RM bench press (kg) and 1RM leg press (kg) tests

[b]*P* interaction <0.20

PHV, and TBLH LM. Our results were comparable to a longitudinal study in which serum VEGF was inversely associated with BMC/height at the total body in boys with overweight whose BMI gain was higher during pubertal years.[11] Elevated circulating levels of VEGF have been found in obese population as hypoxia-induced by adipose tissue expansion produce VEGF.[28] However, VEGF functions on bone development depend both on autocrine and paracrine pathways. For instance, VEGF stimulates osteoblast differentiation and inhibits adipocytes differentiation via intracrine pathway, whereas osteoblast-derived VEGF leads osteoclast differentiation via paracrine pathway.[29] In the light of these findings, we could speculate that despite VEGF concentrations are important for the adipose tissue vascularization in this population, highly expressed VEGF as a consequence of the overweight/obese condition might have detrimental effects on bone mass accumulation, possibly explained by the dysregulation of autocrine and paracrine mechanisms.

IL-6 was also inversely associated with TBLH BMC after controlling for the same set of cofounders. Our results are in accordance with Hanks et al.,[10] who found a negative correlation between IL-6 and BMC in prepubertal girls. Similarly, Mengel et al.[11] found that the changes in serum IL-6 were negatively correlated with LS aBMD in boys with overweight and extensive BMI gain during the pubertal years. However, they did not find any association between IL-6 and LS aBMD after controlling for the effect of testosterone, body fat percentage, and BMI. Previous studies have reported that IL-6 directly promotes osteoclastogenesis by binding with receptors on pre-osteoclasts or indirectly alters bone remodeling by inducing JAK/STAT3 pathways through osteoblasts and secrete pro-osteoclasts mediators (i.e., receptor activator of nuclear factor κ-B ligand [RANKL] and IL-1).[30] Overall, our findings agree with the idea that IL-6 have anti-osteogenic and pro-osteoclastic effects on bone and these effects might already be present in prepubertal children with overweight/obesity.

Like IL-6, in vitro studies have also reported the osteoclastogenic role of TNF-α.[31] Otherwise, few studies have documented this role in humans. Zheng et al.[32] reported that TNF-α produced by stimulated whole blood cells was inversely associated with LS aBMD in postmenopausal women, whereas Ding et al.[33] found that the inverse association between serum TNF-α and LS aBMD in

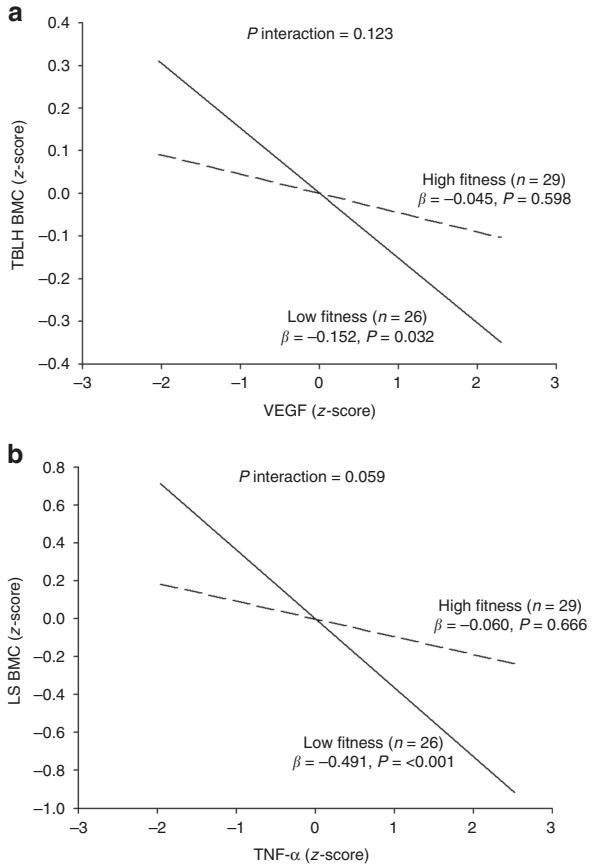

Fig. 2 Graphical representation of the standardized regression slopes between VEGF and TBLH BMC by levels of muscular fitness (**a**) and between TNF-α and LS BMC by levels of muscular fitness (**b**). High/low-fitness groups were defined as being above/below the age, sex, and study-specific median values for average muscular fitness *z*-score (*z*-score mean was computed from 1RM bench press (kg) and 1RM leg press (kg) tests). The regression models were adjusted for sex, years from PHV, and TBLH LM. The standardized coefficients are interpreted as the number of SDs that the outcome changes as a result of 1-SD change in the predictor. PHV, peak height velocity; VEGF, vascular endothelial growth factor A; TNF-α, tumor necrosis factor-α; TBLH, total body-less head; BMC, bone mineral content; LM, lean mass

older men disappeared after controlling for IL-6. Our results indicate that plasma levels of TNF-α were inversely associated with LS BMC independently of sex, years from PHV, and TBLH LM. Besides, IL-6 did not come up as a predictor of LS BMC in the stepwise multiple linear regression.

IL-1β is a proinflammatory marker associated with osteoclastogenesis via induction of RANKL and inhibition of osteoprotegerin and reduces osteoblast recruitment in vitro.[34] Unexpectedly, our results suggest, for the first time, a positive association between IL-1β and LS BMC after controlling for potential cofounders in children with overweight/obesity. This finding agrees with the study of Pacifici et al.,[35] in which an increased IL-1 production was associated with bone formation in adults independently of sex and menopausal status. This can be explained by the fact that short stimulation of mesenchymal stem cells with IL-1β leads to osteogenic differentiation through the upregulation of genes in MG63-GFP osteoblasts.[36] On the contrary, IL-1β has been negatively associated with LS aBMD in postmenopausal women[32] and Mengel et al.[11] did not find significant associations between IL-1β and aBMD in overweight children.

Muscular fitness, inflammatory markers, and bone mass in overweight/obese children

Bone marrow is a complex environment, in which a variety of cell types (i.e., blood cells, osteoblasts, osteoclasts, and adipocytes) share a common space locally releasing cytokines and growth factors that could affect the cells in their proximity.[8] Furthermore, bone development is regulated by modeling and remodeling processes that depend on the mechanical forces applied by the muscles to the skeleton.[37] Our study suggests that higher levels of muscular fitness might attenuate the detrimental effects of VEGF and TNF-α on TBLH BMC and LS BMC, respectively (Fig. 2). In this regard, partial correlations controlling for sex and years from PHV showed that VEGF was negatively correlated with muscular fitness (data not shown, $r = -0.36$, $P = 0.008$), although no evidence of correlation was found between TNF-α and muscular fitness (data not shown, $r = -0.01$, $P = 0.923$). The latter result contrasts with Steene-Johannessen et al.,[19] who found a negative correlation between TNF-α and muscular fitness in prepubertal children after controlling for pubertal stage. Nevertheless, the beneficial effect of muscular fitness on bone development is well documented in growing children.[38] Moreover, the association between muscular fitness and bone mass is mediated by LM in prepubertal children.[15] Likewise, an inhibitory effect of LM on obesity-related inflammation has been suggested in middle-aged adults.[39] Thereby, our findings agree with the literature and support the fact that there is a crosstalk between adipocytes and myocytes interacting with obesity and its related disorders even in children with overweight/obesity. We speculate that the detrimental consequences of excessive FM (i.e., inflammation) in children with overweight/obesity could be counteracted, to some extent, by maintaining optimal levels of muscular fitness.

Strengths and limitations

Some limitations need to be considered. At first, our cross-sectional design rules out the possibility of identifying cause–effect relationships. Secondly, the number of participants with complete data in all studied variables is relatively small, but similar to previous studies.[11,12] Thirdly, our study has used plasma samples to measure inflammatory markers. Previous studies have used plasma or serum samples and therefore, comparisons may be affected. However, as shown in a recent 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.[40] Notwithstanding, the use of DXA and the accuracy of the objective methodology used for muscular fitness and blood measurements are strengths of this study.

CONCLUSION

In summary, our findings suggest that the link between obesity and bone health may be at least explained by inflammatory mechanisms in children with overweight/obesity. Specifically, IL-6 and VEGF were negatively associated with TBLH BMC, whereas TNF-α (negatively) and IL-1β (positively) were associated with LS BMC. Furthermore, our data suggest that high levels of objectively measured muscular fitness may attenuate the adverse effects of VEGF and TNF-α on TBLH BMC and LS BMC, respectively. In the light of these findings, appropriate levels of muscular fitness may preserve normal bone accretion in this population. Future longitudinal and intervention studies in this population are needed to confirm these findings.

ACKNOWLEDGEMENTS

This study was supported by the Spanish Ministry of Economy and Competitiveness (Reference DEP2013-47540). This study took place owing to the additional funding from the University of Granada, Plan Propio de Investigación 2016, Excellence actions:

Units of Excellence; Unit of Excellence on Exercise and Health (UCEES) and by the Junta de Andalucia, Consejeria de Conocimiento, Investigación y Universidades and the European Regional Development Funds (ref. SOMM17/6107/UGR). In addition, this study was further supported by the SAMID III network, RETICS, funded by the PN I + D + I 2017-2021 (Spain), ISCIII- Sub-Directorate General for Research Assessment and Promotion and the European Regional Development Fund (ERDF) (Ref. RD16/0022). M.A.-R. is supported by a Predoctoral Research Grant from Universitat Jaume I (PREDOC/2015/13). J.M.-G. is supported by the Spanish Ministry of Education, Culture, and Sport (FPU14/06837). C.C.-S. is supported by a grant from the Spanish Ministry of Economy and Competitiveness (BES-2014-068829); and by the Government of Andalusian, Integrated Territorial Initiative 2014–2020 for the province of Cádiz (PI-0002-2017). A.P.-F. is supported by the Spanish Ministry of Education, Culture, and Sport (FPU 16/02760). L.G.-M. is funded by Plan Propio de Investigación 2016 (Programa de Captación de Talento and Programa de Captación de Talento—UGR Fellows). This work is part of a Ph.D. thesis conducted in the Biomedicine Doctoral Studies at the University of Granada, Spain. Portions of this study were presented at Symposium EXERNET. Research in physical exercise and health: Exercise is medicine, October 19–20, 2018, Pamplona, Spain.

AUTHOR CONTRIBUTIONS

J.J.G.-C. was involved in the data collection and analysis, and drafting the manuscript. C.C.-S., J.M.-G., A.P.-F., C.M.A., J.G.-V., J.M. and F.B.O. were involved in the study design, data collection, and critical revision. L.G.-M., E.U.-G. and F.B.O. takes responsibility for the integrity of the data analyses. L.G.-M., E.U.-G., I.L., J.J. and F.B.O. participated in the interpretation of the results and critical revision. M.A.-R. was involved in the manuscript preparation and critical revision. All authors have read and approved the final version of the submitted manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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