High Adiposity and Serum Leptin Accompanied by Altered Bone Turnover Markers in Severe Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objective.*** To evaluate interactions between skeleton and adipose tissue, and association of adipokines and bone turnover markers with disease-related factors in patients with severe juvenile idiopathic arthritis (JIA).

Methods. Forty-nine patients (median age 14.8 yrs, median disease duration 10.2 yrs) with refractory polyarticular JIA and 89 sex-matched and age-matched healthy controls participated in the study. Study subjects underwent clinical examination, body composition assessment with dual-energy X-ray absorptiometry, and analyses for leptin, adiponectin, and bone turnover markers.

Results. Patients with JIA were shorter and more often overweight ($p = 0.001$) or obese ($p < 0.001$) than controls. They had significantly higher serum leptin, even when adjusted for fat mass ($p < 0.001$), than did controls. Adiponectin did not differ between the groups. Concentration of carboxyterminal telopeptide of type I collagen was higher ($p = 0.006$) in patients. The inverse association between leptin and bone turnover markers disappeared in controls but was strengthened in patients when adjusted for fat mass. Leptin, adiponectin, or bone markers did not associate with variables of disease activity.

Conclusion. Patients with severe JIA had high adiposity accompanied by increased bone resorption. Their serum leptin was higher, even independently of fat mass. Leptin tended to associate inversely with bone turnover markers but did not associate with variables of disease activity. (First Release Oct 15 2014; J Rheumatol 2014;41:2474–81; doi:10.3899/jrheum.131107)

Key Indexing Terms:
JUVENILE IDIOPATHIC ARTHRITIS FAT MASS ADIPOKINES
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Childhood rheumatic diseases, including juvenile idiopathic arthritis (JIA), may impair bone mass accrual and result in increased fractures from proinflammatory cytokines, glucocorticoid (GC) therapy, delayed puberty, malnutrition, low muscle mass, and physical inactivity[1]. Obesity may be an additional risk factor for impaired bone health[2]. In children, obesity is associated with low bone mineral density for body size[3], impaired skeletal mechanical properties[4], and increased number of fractures[5,6]. Studies show that vertebral fractures in pediatric rheumatic diseases are associated with overweight/obesity[7,8,9]. The mechanisms explaining the relationship between obesity and bone health in childhood are poorly understood. In addition to loading, the metabolic situation seems crucial.

White adipose tissue produces numerous adipokines that participate in the regulation of several physiological processes including the endocrine system, immunity, inflammation, and bone turnover[2,10]. Body fat content is strongly associated with serum leptin[10,11], of which the effects are primarily proinflammatory[10,12]. Adiponectin is inversely related to body fat and is mostly associated with antiinflammatory effects; low adiponectin concentrations are observed in visceral obesity, insulin resistance, and

cardiovascular diseases[10]. However, adiponectin may also have proinflammatory properties, and certain chronic inflammatory diseases including rheumatoid arthritis (RA) are associated with increased adiponectin[13,14,15].

Adipokines are increasingly recognized as mediators of systemic and local inflammation in RA[12]. In most clinical studies, increased serum leptin levels are associated with elevated C-reactive protein (CRP) and increased disease activity[13,15,16]. Studies show increased serum adiponectin levels[13,14,15,16] that are positively associated with progression of radiological joint destruction[17,18]. However, adiponectin correlates inconsistently with disease activity and CRP[13,14,15,16].

Leptin is involved in the regulation of bone homeostasis[19]. In rodent models, leptin inhibits osteoblast formation indirectly through the central nervous system[20], but also has anabolic effects on osteoblasts locally in the bone microenvironment[21]. Pediatric studies report discordant effects of leptin on bone turnover markers (BTM)[11,22,23,24]. *In vitro* studies show both positive and negative effects of adiponectin on bone formation[25,26]. No correlations between adiponectin and BTM appear in healthy lean or obese children[11,24]. The effect of childhood overweight on bone metabolism seems controversial[11,22,23,24,27]. Previous JIA studies report alterations in BTM, mostly reduced bone formation often in association with active disease[28,29]. Results on bone resorption are more conflicting[28,29].

Obese patients with rheumatic diseases are exposed to complicated interactions between adipose tissue, inflammation, and bone metabolism. We previously observed that vertebral fractures are related to high body mass index (BMI) in refractory polyarticular JIA[9]. In this cohort, our primary aim was to explore the effect of fat mass and adipokines on BTM in comparison with healthy controls. We hypothesized that adipokine values would be associated with BTM, and anticipated patients with JIA to have increased leptin and consequently decreased bone formation and/or increased bone resorption. Secondly, we explored associations between disease activity, adipokines, and BTM. We are not aware of previous data concerning these issues in JIA.

MATERIALS AND METHODS

Study population. The study was carried out at Heinola Rheumatism Foundation Hospital, a tertiary center treating complicated pediatric rheumatology cases in Finland. The study protocol was approved by the Helsinki University Central Hospital Ethics Committee, and written informed consent was obtained from all participants and/or their parents. Participants were originally recruited for a study evaluating the prevalence and risk factors of compression fractures in severe JIA[9], including children and adolescents with a history of refractory disease with continuous disease activity or recurrent flares requiring permanent antirheumatic medication since JIA diagnosis. Patients were diagnosed according to the revised criteria[30]. The inclusion criteria were (1) age < 19 years, (2) polyarticular JIA (polyarthritis, extended oligoarthritis, or psoriatic arthritis with ≥ 5 affected joints) for at least 5 years, or (3) systemic arthritis for at least 3 years. Fifty of the 55 recruited consecutive patients fulfilling the inclusion criteria consented. Nonparticipants did not differ significantly from participants[9]. One girl with JIA was excluded because of extremely short stature (height Z-score –9.4). The final cohort comprised 49 subjects. The study was cross-sectional including anthropometry and clinical assessment, questionnaires, laboratory measurements, bone age assessment, and body composition measurement by dual-energy x-ray absorptiometry (DEXA), all during 1 study visit.

Medical records were reviewed for disease and treatment characteristics. Total duration of GC treatment and a 3-year cumulative systemic GC dose for recent GC exposure, as prednisolone equivalents, were determined. The clinical evaluation was performed by a pediatric rheumatologist. Height and weight were recorded. BMI (kg/m^2) Z-scores were calculated according to reference data (www.who.int), and cutoff values > +1 SD and > +2 SD were used for overweight and obesity. Pubertal maturation was assessed according to Tanner[31]. Number of active joints (including swollen and/or tender joints with limited range of motion), global assessment of overall well-being by parents or patients aged > 15 years, physician's global assessment of disease activity, and Childhood Health Assessment Questionnaire were recorded. Juvenile Arthritis Disease Activity Score in 71 joints was calculated[32]. Inactive disease while taking medication was defined according to Wallace, *et al*[33]. Leisure time physical activity and participation in physical education at school were assessed with a questionnaire.

Control subjects. For each patient, 1–2 controls of same sex and similar age were selected from a representative cohort of apparently healthy Finnish schoolchildren from Helsinki district[27]. The original cohort comprised 202 children and adolescents from 7 to 19 years of age; 62% were girls. There were 89 matching controls, 2 for each boy and 1.7 for each girl. Their background characteristics and medical history were collected with questionnaires and interview. Anthropometry, body composition measured with DEXA, and laboratory samples were obtained during the study visit. Assessment of pubertal maturation was based on serum gonadotropin and sex steroid concentrations. Laboratory samples of controls and patients were analyzed similarly.

Imaging studies. Bone age was determined for the patients from a plain radiograph of the left hand by a pediatric endocrinologist (OM)[34]. It was considered delayed (42%) or advanced (6%) when it differed from the chronological age by more than 1 year. Bone age was used instead of chronological age in all analyses for patients. Body composition was assessed by DEXA (for patients, Lunar Prodigy; GE Lunar; for controls, Hologic Discovery A). Because BMI has limitations as an indicator of adiposity, we used fat mass, calculated from DEXA-derived fat percentage, for statistical analyses. Data on cross-calibration by Shepherd, *et al* were used to transform Hologic fat percent values comparable to those of Lunar[35].

Laboratory measurements. Morning blood samples were collected after an overnight fast and stored at –80°C until analyzed. Serum leptin and adiponectin concentrations were assessed using human leptin and adiponectin ELISA (R&D Systems). Serum concentrations of aminoterminal propeptides (PINP), and carboxyterminal telopeptides (ICTP) of type I collagen were assessed by radioimmunoassay (Orion Diagnostica). Serum total alkaline phosphatase (ALP) was measured with a standard kinetic method (Roche Diagnostics). Serum total osteocalcin (OC) was determined by 2-site immunoassay based on monoclonal antibodies[36,37]. All samples were measured as duplicates at the end of the study. Girls aged > 8 years and boys > 10 years were assessed for gonadotropin (follicle-stimulating hormone, luteinizing hormone) and sex steroid (estradiol or testosterone) concentrations. Erythrocyte sedimentation rate and CRP were assessed for patients.

Statistical analysis. Statistical analyses were performed with the SPSS for Windows, version 17.0 (SPSS Inc.). Comparisons between the 2 groups were performed with independent samples t-test, or, in case of non-normally distributed values, with Mann-Whitney U test; chi-square

test or Fisher's exact test were used for categorical variables. Pearson correlation was used to identify associations between variables. Ln-transformations were used for non-normally distributed values. Multivariate analysis of covariance was used to compare concentration of adipokines and BTM between groups when adjusting for confounders: age, sex, pubertal development, and fat mass. An interaction between case status and fat mass on leptin ($p = 0.002$) was observed with analysis of covariance. Based on this, associations of adipokines (exposures) on BTM (outcomes) were analyzed in the 2 groups separately with linear regression. The associations were explored in 4 additive models: unadjusted; and adjusted for age, sex, and pubertal status; fat mass; and lean mass. In patients with JIA, a further adjustment for the current weight-adjusted GC dose was performed. We applied Bonferroni adjusted p values to avoid errors related to multiple testing. Thus, the required significance level depended on the number of predictors in the model: unadjusted ($p < 0.05$); adjusted for age, sex, and pubertal status ($p < 0.0125$); fat mass ($p < 0.01$); lean mass ($p < 0.008$); and GC dose ($p < 0.007$). Five patients with previous bisphosphonate therapy were excluded from all analyses involving BTM; their fat mass, BMI, leptin, or adiponectin concentrations did not differ from the other patients.

RESULTS

Characteristics of the study population. The study included 49 patients (40 females) with a median age of 14.8 years (range 7.0–18.7) and median disease duration of 10.2 years (3.9–16.8). Disease characteristics and data on medication are presented in Table 1. Most of the patients were taking systemic GC and/or received biological therapy (65% had a history of ≥ 2 biological drugs). All patients had received intraarticular GC injections (up to several hundred), 21 patients had used ocular steroids, and 5 patients inhaled steroids. Altogether 96% of the patients had active disease while taking medication[33].

Age, sex distribution, and pubertal maturation were similar in patients and controls; > 50% in both groups were postpubertal. Patients were shorter, had higher fat mass, and were more often overweight or obese than controls; lean mass was similar in both groups (Table 2). Physical activity was assessed with different questionnaires in patients and controls, and no direct comparisons between the groups could be made, but 53% of the patients and none of the controls had limited participation in school physical education. Patients' leisure time sport activities were largely non–weight-bearing such as swimming, cycling, and riding (48%).

Biochemical findings. Leptin correlated with BMI in both controls and patients ($r = 0.61$ and 0.73, respectively, $p < 0.001$), and similarly with fat mass ($r = 0.75$ and 0.74, $p < 0.001$). Patients had significantly higher leptin concentrations than did controls (Table 3). Leptin concentration remained higher in patients even when adjusted for age, sex, pubertal development, and fat mass (19.9, 95% CI 17.4–22.5 vs 11.8, 95% CI 10.0–13.7; $p < 0.001$; Figure 1). The β coefficient for the association between fat mass and leptin remained constant in controls but became stronger in patients when adjusted for sex, age, and lean mass. Introducing height in the model had a minor effect on results but caused issues with multicollinearity.

Table 1. Disease characteristics and medication of the 49 patients with JIA.

Characteristic	Median (IQR) or n (%)
Age at time of study, yrs	14.8 (12.2–16.3)
Age at diagnosis, yrs	2.3 (1.7–5.5)
Disease duration, yrs	10.2 (7.4–13.5)
JIA subtype	
Rheumatoid factor-negative polyarthritis	27 (55)
Rheumatoid factor-positive polyarthritis	1 (2)
Oligoarthritis, extended	14 (29)
Psoriatic arthritis	1 (2)
Systemic arthritis	6 (12)
History of uveitis	21 (43)
Antinuclear antibody-positive	13 (27)
Disease activity	
No. active joints	1 (0–3.5)
Physician's global assessment*	19 (10–36)
Parent's global assessment*	15 (4–40)
Childhood Health Assessment Questionnaire	0.125 (0–0.5)
Erythrocyte sedimentation rate, mm/h	11 (7–19)
JADAS-71	5.8 (2.5–8.7)
CRP, mg/l	5 (5–5)
Medication at time of study	
NSAID regularly	8 (16)
Methotrexate	28 (57)
Hydroxychloroquine	14 (29)
Sulfasalazine	6 (12)
Azathioprine	9 (18)
Leflunomide	12 (24)
Other**	3 (6)
Biological therapy ever	46 (94)
Biological therapy at time of study	44 (90)
Etanercept	23
Infliximab	8
Adalimumab	12
Anakinra	1
Systemic GC ever	46 (94)
Systemic GC at time of the study	25 (51)
Duration of GC therapy, yrs	7.0 (4.4–10.4)
Cumulative GC dose for the 3 preceding yrs, g***	3.2 (0.3–5.6)
Weight-adjusted 3-yr cumulative GC dose, mg/kg***	69 (5.5–128)

* Visual analog scale (0–100 mm). ** Cyclosporine A (n = 1), sodium aurothiomalate (1), thalidomide (1). *** Includes oral and intravenous GC. JIA: juvenile idiopathic arthritis; IQR: interquartile range; CRP: C-reactive protein; JADAS-71: Juvenile Arthritis Disease Activity Score in 71 joints (score range 0–101); NSAID: nonsteroidal antiinflammatory drugs; GC: glucocorticoid.

Adiponectin did not correlate with fat mass in either group. In patients, but not in controls, adiponectin correlated with lean mass ($r = –0.36$, $p = 0.010$). Adiponectin concentrations did not differ between groups (Table 3).

The resorption marker ICTP was significantly higher in patients while concentrations of OC, ALP, and PINP did not differ between the groups, suggesting imbalance in bone turnover in subjects with JIA (Table 3).

Interactions between fat mass or adipokines and BTM. In healthy controls, all BTM were inversely associated with fat mass (for OC r –0.58; PINP r –0.55; ALP r –0.50; ICTP r –0.44; $p < 0.001$ for all). After adjusting for age, sex,

Table 2. Anthropometric data for patients with JIA and healthy controls, presented as mean ± SD, unless otherwise indicated.

	Controls, n = 89	Patients, n = 49	p
Age, yrs	13.9 ± 3.0	14.2 ± 3.0	0.569[1]
Female, n (%)	71 (79.8)	40 (81.6)	0.792[3]
Height, cm	155.6 ± 13.6	149.6 ± 12.8	**0.005**[2]
Height, Z-score	0.0 ± 0.9	–1.3 ± 1.2	**< 0.001**[1]
Weight, kg	47.8 ± 13.3	52.9 ± 16.8	0.189[2]
Height-adjusted weight, %	6.6 ± 15.9	33.1 ± 34.7	**< 0.001**[2]
BMI, kg/m²	19.3 ± 3.2	23.4 ± 6.0	**< 0.001**[2]
BMI, Z-score	–0.1 ± 1.1	1.0 ± 1.5	**< 0.001**[1]
Overweight/obese, n (%) *	13 (15.1)	19 (38.8)	**0.001**[3]
Obese, n (%) **	1 (1.1)	14 (28.6)	**< 0.001**[4]
Fat, %	29.0 ± 7.6	35.1 ± 10.1	**< 0.001**[1]
Fat mass, kg	14.2 ± 6.9	20.1 ± 10.5	**< 0.001**[2]
Lean mass, kg	31.4 ± 8.1	30.9 ± 7.8	0.442[2]
Puberty, n (%)			0.442[3]
Prepubertal	22 (24.7)	8 (16.3)	
Pubertal	21 (23.6)	15 (30.6)	
Postpubertal	46 (51.7)	26 (53.1)	

* BMI Z-score > +1.0 SD; ** BMI Z-score > +2.0 SD. P values refer to differences between the patients and controls and were calculated by t-test[1], Mann-Whitney U test[2], chi-square test[3], or Fisher's exact test[4], as appropriate. Statistically significant values are marked in bold. JIA: juvenile idiopathic arthritis; BMI: body mass index.

Table 3. Biochemical findings in patients with JIA and controls, presented as mean ± SD.

	Controls, n = 89	Patients, n = 44	p
S-Leptin, ng/ml	8.9 ± 7.6	25.4 ± 19.0	**< 0.001**
S-Adiponectin, μg/ml	10.6 ± 4.6	10.1 ± 4.7	0.708
S-OC, μg/l	24.9 ± 14.2	27.5 ± 14.4	0.274
S-ALP, U/l	156 ± 87	152 ± 101	0.550
S-PINP, μg/l	373 ± 306	426 ± 292	0.190
S-ICTP, μg/l	12.3 ± 5.5	15.6 ± 6.8	**0.010**

P values were calculated with Mann-Whitney U test . Statistically significant values are marked in bold. JIA: juvenile idiopathic arthritis; S-OC: osteocalcin; S-ALP: alkaline phosphatase; S-PINP: procollagen type I aminoterminal propeptide; S-ICTP: carboxyterminal telopeptide of type I collagen.

pubertal status and lean mass, the p values were 0.001, 0.011, 0.119, and 0.151, respectively. Conversely, in patients, fat mass and BTM were not related.

We further tested to see whether BTM was associated with leptin or adiponectin in different models (Table 4). In controls, significant inverse associations were observed between leptin and all BTM. The associations of leptin with BTM weakened after adjustments, especially for fat mass, while in patients no associations were present between leptin and BTM in the unadjusted model. However, correcting for fat mass substantially strengthened the inverse association between leptin and BTM. After considering Bonferroni-adjusted p values, these associations were not significant except for PINP. Introducing lean mass in the model induced a decrease in β-coefficient values. Adiponectin was not associated with any BTM in either group.

Interactions of disease-related factors with adiposity, adipokines, and BTM. We did not observe significant correlations between fat mass and disease activity, disease duration, or the recent or current weight-adjusted GC dose. Leptin and adiponectin did not correlate with any of these variables. The inverse correlations between the current weight-adjusted GC dose and BTM were not significant, and the current GC exposure had a minor effect on the relationships between adipokines and BTM (Table 4).

DISCUSSION

Data on fat-bone interactions and the role of adipokines in pediatric rheumatology are very limited. We evaluated interactions of fat mass and adipokines with bone metabolism in 49 patients with severe JIA and in 89 healthy controls. We observed higher leptin concentrations in patients, even after

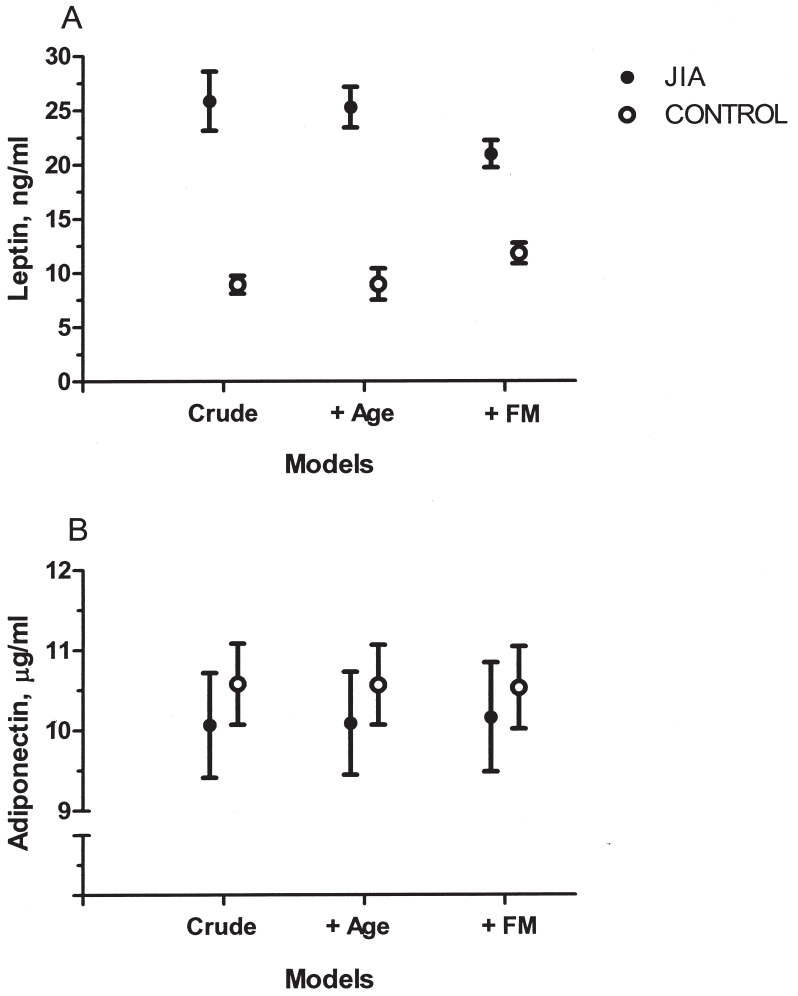

Figure 1. Comparison of serum leptin (A) and adiponectin (B) values between the groups when (1) unadjusted, (2) adjusted for age, sex, and pubertal stage, and (3) adjusted additionally for fat mass (FM). JIA: juvenile idiopathic arthritis.

correcting for fat mass, and leptin tended to be inversely associated with BTM. The bone resorption marker ICTP was increased in patients. No significant correlations between disease activity and adipokines or BTM were observed.

Previous studies on JIA report low or normal BMI[29,38,39], but more recent studies show increased fat mass and BMI[40,41]. In line with these recent observations our patients had increased body fat. The global phenomenon of increasing obesity certainly affects also patients with JIA but would not explain the higher degree of obesity in patients than in controls. Our patients had longstanding active disease with complex medication. Prolonged GC therapy increases body fat mass and enhances fat deposition especially to the visceral compartment, while reducing peripheral fat stores[42]. Fifty-one percent of our patients were currently taking systemic GC and only 6% were unexposed. Before the era of biological drugs, a study from our hospital reported normal BMI in a cohort with polyarticular JIA and GC therapy[39]. Altogether 94% of our patients were exposed to biological drugs, often to several preparations. Adult studies suggest weight gain with anti-tumor necrosis factor (TNF)-α therapy[43]. The possible effects of biological drugs on appetite or metabolism deserve further studies in all age groups. Physical inactivity may also contribute to weight gain because half of our patients had limited participation in physical education at school and their leisure time sport activities were often non-weight-bearing.

The lack of differences in bone formation markers between patients and controls may be related to the relatively low disease activity at the time of the study in the majority of the patients. However, because their ICTP values were higher, increased resorption with unaltered formation suggest imbalanced bone turnover. In adults with RA, increased serum ICTP levels have been associated with

Table 4. Linear regressions between bone turnover markers and leptin and adiponectin in controls and patients with JIA with standardized β coefficients and p values.

	Controls, n = 89								Patients with JIA, n = 44							
	OC		ALP		PINP		ICTP		OC		ALP		PINP		ICTP	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Leptin																
Unadjusted	**–0.514**	**< 0.001**	**–0.467**	**< 0.001**	**–0.519**	**< 0.001**	**–0.448**	**< 0.001**	–0.197	0.200	–0.043	0.783	–0.132	0.394	–0.082	0.597
Adjusted*	**–0.250**	**0.006**	–0.125	0.128	**–0.202**	**0.011**	–0.150	0.098	–0.213	0.077	–0.048	0.663	–0.143	0.186	–0.097	0.400
+ Fat mass	–0.076	0.522	–0.057	0.604	–0.130	0.219	–0.114	0.351	–0.495	0.016	–0.410	0.025	**–0.513**	**0.004**	–0.438	0.025
+ Lean mass	–0.033	0.798	–0.046	0.705	–0.082	0.466	–0.070	0.606	–0.327	0.129	–0.315	0.112	–0.371	0.048	–0.284	0.167
+ GC									–0.291	0.168	–0.280	0.148	–0.349	0.063	–0.265	0.201
Adiponectin																
Unadjusted	–0.045	0.674	–0.003	0.975	–0.029	0.787	–0.115	0.283	0.066	0.670	0.110	0.479	0.057	0.715	0.060	0.700
Adjusted*	–0.092	0.267	–0.032	0.667	–0.075	0.294	–0.170	0.035	–0.217	0.097	–0.208	0.075	–0.272	0.017	–0.244	0.046
+ Fat mass	–0.090	0.245	–0.031	0.672	–0.074	0.286	–0.169	0.035	–0.241	0.074	–0.191	0.110	–0.276	0.020	–0.241	0.058
+ Lean mass	–0.091	0.258	–0.032	0.669	–0.049	0.490	–0.151	0.072	–0.143	0.286	–0.130	0.293	–0.192	0.100	–0.156	0.221
+ GC									–0.162	0.214	–0.149	0.213	–0.206	0.075	–0.168	0.188

Parenthesis for ln-transformed variables. * Adjusted for age (controls) or bone age (patients), sex, and pubertal status. GC: current weight-adjusted glucocorticoid dose. Statistically significant Bonferroni adjusted p values are marked in bold. JIA: juvenile idiopathic arthritis; OC: osteocalcin; PINP: procollagen type I aminoterminal propeptide; ICTP: carboxyterminal telopeptide of type I collagen; ALP: alkaline phosphatase.

disease activity and radiological progression, reflecting pathological matrix metalloproteinase-mediated degradation of type I collagen[44]. However, ICTP did not correlate with disease activity in our patients. Although GC can affect bone resorption[45], we observed no significant correlation with recent or current GC dose.

Fat mass was inversely associated with OC and PINP in our controls, in line with earlier studies reporting low OC values in overweight children[22,23]. In the case of ICTP, significance was lost after adjustments. Increased carboxyterminal collagen crosslinks (CTX) levels in obese children have been reported[11]. It cannot be excluded that methodological differences may affect the results. ICTP and CTX are both C-terminal telopeptides of type I collagen, but they are produced by different biological pathways; ICTP is cleaved by matrix metalloproteinases, whereas CTX is produced by cathepsin K-mediated resorption[46]. In patients, no associations occurred between fat mass and any of the BTM.

To our knowledge the only previous study assessing leptin in JIA reported low BMI and low serum leptin, while leptin-to-BMI ratio was comparable with controls[38]. Our patients had significantly higher adiposity and significantly higher serum leptin versus controls, even when adjusted for fat mass. Controlling for lean mass strengthened the association between fat mass and leptin in patients. Introducing lean mass in the model removed confounding factors such as visceral fat and allowed us to observe a stronger association between fat mass and leptin. The interaction noted in the groups suggests that, while leptin is higher overall in JIA, the greatest differences in leptin levels between JIA and controls are among those with low fat mass. Similar to some and opposite to other studies[11,22,23,24], leptin and BTM were inversely associated in our controls. However, these associations appeared to be dependent on fat mass. In patients, adjustment for fat mass substantially strengthened the inverse association between leptin and BTM, especially PINP. Even though these associations attenuated after correcting for lean mass in patients, the results remained different from the controls. This implies that leptin may play a role in suppressing bone turnover in JIA through mechanisms other than increased fat mass. In addition, as the central and peripheral effects of leptin on bone formation are opposite based on experimental data[20,21], our results may mirror differences in the site of leptin production and the degree of leptin resistance between patients and controls.

Data suggest that obesity is associated with increased disease activity in RA[47], but 1 study found no association in JIA[41]. We did not observe significant correlations between fat mass and disease activity. Proinflammatory and immunomodulatory actions of leptin have been recognized in rheumatic diseases[12], yet studies on the role of leptin in pediatric rheumatic diseases are sparse. Al and co-workers found no correlation between serum leptin and disease activity in pediatric systemic lupus erythematosus (SLE)[48], similar to our patients with severe JIA.

We are not aware of any previous data regarding the role of adiponectin in JIA. Adiponectin did not correlate with fat mass in either group. In adults with RA, high serum adiponectin has been observed especially in individuals with low visceral fat content and high radiological damage scores[12]. Considering the significantly higher adiposity but still not lower adiponectin levels in our patients, chronic inflammation may have an effect. However, we did not find any significant associations between adiponectin and variables of disease activity, in line with observations in

pediatric SLE[48]. No associations were observed between adiponectin and BTM in either group. Whether these findings are related to other metabolic factors or modified by disease-related and treatment-related factors remains to be elucidated.

We recognize certain limitations in our study. The rather small study cohort may have prevented us from observing some biologically significant associations. Patients with JIA had longstanding refractory disease, with complex medication possibly affecting our results. Systemic GC can affect bone turnover marker concentrations[45], but their effects on adipokines are not as well characterized[49]. Also, data on the effects of TNF-α blockers on adipokines[12,49] and bone turnover markers[50] are inconsistent. Physical activity was not similarly assessed in the 2 groups and thus could not be compared. Cross-calibration of DEXA results is a possible source of bias because we used software versions older than those used in the original study by Shepherd, *et al*[35]. The DEXA findings were in line with the significant differences in leptin and BMI between the groups, but it is possible that the applied adjustments affected the magnitude of fat mass differences between the groups. Further, we were unable to evaluate fat distribution and quantify visceral fat. Our study would have been strengthened by more thorough evaluation of metabolic variables and additional markers of inflammation and bone resorption.

We found increased adiposity and circulating leptin accompanied by increased bone resorption marker ICTP in a cohort of children and adolescents with refractory polyarticular JIA. Circulating leptin was higher even when corrected for fat mass in patients, and tended to be inversely associated with BTM, which are novel findings in JIA. Further longitudinal studies are needed to evaluate causal relationships and to elucidate the possible contributing effects of antiinflammatory and antirheumatic drugs. Future evaluations are also warranted to differentiate between the effect of JIA-associated inflammatory and autoinflammatory factors, and adipose tissue-derived inflammation on BTM. Ideally these should be carried out in BMI-matched patient and control groups and in cohorts with normal fat mass. The findings do, however, support the significance of weight control for optimal bone health in patients with JIA.

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