

The Interrelationships between Abdominal Adiposity, Leptin and Bone Mineral Content in Overweight Latino Children

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Key Words

Abdominal adiposity · Leptin · Osteopenia · Bone mineral content

Abstract

Background/Aims: The link between abdominal fat and bone mineral content (BMC), independent of weight, has not been extensively studied. In Latino children, the contributions of abdominal subcutaneous and visceral fat to BMC have not been examined. Research on the effect of leptin on BMC has also been inconclusive. **Methods:** The present study included 256 overweight Latino children (111 girls, 145 boys; mean BMI 28.2; age 11.1 ± 1.7 years) from Los Angeles, California. Subcutaneous abdominal adipose tissue (SAAT) and intra-abdominal adipose tissue (IAAT) were determined by single-slice magnetic resonance imaging. BMC was measured using dual-energy X-ray absorptiometry. **Results:** Independent of age, Tanner stage and weight, abdominal adipose tissue (SAAT + IAAT) was inversely correlated with BMC ($r = -0.46$, $p < 0.0001$; $n = 256$). In girls, there was an inverse correlation between SAAT and BMC ($r = -0.38$, $p < 0.05$), between IAAT and BMC ($r = -0.32$, $p < 0.05$) and between leptin and BMC ($r = -0.39$, $p < 0.05$). In boys, SAAT and BMC were inversely correlated ($r = -0.26$, $p < 0.05$), but the correlation between IAAT and BMC was not significant ($p = 0.22$). Leptin was also inversely correlated with BMC ($r = -0.38$, $p < 0.05$)

in boys and contributed to the variances in BMC in both girls and boys. **Conclusion:** Total abdominal adipose fat and leptin are negatively associated with BMC in Latino children. The correlation between SAAT and BMC is stronger in girls than boys. IAAT and BMC are negatively associated in girls but not correlated in boys. Copyright © 2009 S. Karger AG, Basel

Introduction

Obesity has reached epidemic proportions globally and is well-documented. Central adiposity poses a major risk for chronic diseases such as hypertension, stroke and diabetes [1, 2]. An association between central adiposity and osteopenia has also been shown in adults [3] and was recently reported by our group [4] in Caucasian and African-American children. However, the link between central adiposity and bone mineral content (BMC) has not been examined in Latino children, who are especially vulnerable to the health burden of overweight and obesity.

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At similar levels of adiposity, Latino adults have been found to have greater central fat distribution [5] and to be more insulin-resistant [6] than Caucasian adults. Since obesity in adulthood is associated with obesity during childhood [7] and osteoporosis in adulthood is a function of peak bone mass achieved at the end of adolescence [8], understanding the interaction between these two diseases in different ethnic populations of children is of paramount importance. For this reason, the primary objective of this study was to determine whether central adiposity is inversely related to BMC in a population of Latino children. Secondly, we sought to determine which component of central fat, subcutaneous abdominal adipose tissue (SAAT) or intra-abdominal adipose tissue (IAAT), is adversely associated with BMC.

Although previous studies in children [9] have examined the relationship between fat mass and BMC, our previous published work [4] was the first to report the independent contributions of SAAT and IAAT to BMC. Interestingly, these contributions varied across races; while there was a strong inverse correlation between SAAT and BMC in Caucasian children, this relationship was not significant in African-Americans. On the other hand, in African-Americans, IAAT and BMC were inversely correlated, while this relationship was not significant in Caucasians. These racial differences, we concluded [4], may be related to the interactions between ethnicity, body fat distribution, insulin resistance, leptin and bone mass. Given the differences in methodology used to measure SAAT and IAAT in our two studies (computed tomography in the previous study versus MRI in the current study), we are unable to make direct comparisons. In these Latino children, we found a positive relationship between IAAT and insulin resistance [10] and a negative relationship between insulin resistance and BMC [11]. We believe that it is physiologically more plausible that the relationship between central adiposity and BMC in Latinos will be more similar to what we previously observed in African-Americans [4] and hypothesize that IAAT and BMC will be inversely correlated in this cohort.

Methods

Study Cohorts and Subject Description

A cohort of overweight Latino children with a positive family history of type 2 diabetes was established with the intention to follow them longitudinally in the University of Southern California (USC) Study of Latino Adolescents at Risk diabetes project.

A total of 256 children (111 girls, 145 boys) were recruited through clinics, health fairs, newspaper announcements and word of mouth. The children were required to meet the following inclusion criteria: (1) age 8–14 years; (2) body mass index (BMI) \geq 85th percentile for age and sex based on the Centers for Disease Control and Prevention (CDC) standards [12] and an initial telephone prescreening; (3) Latino ancestry (all 4 grandparents Latino by self-report), and (4) family history of type 2 diabetes in at least one parent, sibling or grandparent. The children were of Mexican-American (71%), Central American (16%) or mixed Mexican-Central American (13%) heritage. Children were excluded if they had a prior major illness, including type 1 or 2 diabetes, or took medications or had a condition known to influence body composition or bone mass (e.g. pregnancy, glucocorticoid therapy, hyper- or hypothyroidism). The Study of Latino Adolescents at Risk was approved by the Institutional Review Board, Health Science Campus of USC. Informed consent and assent were obtained from all parents and children, respectively.

Protocol Design

Outpatient Screening Visit. The children arrived at the General Clinical Research Center at approximately 8:00 a.m. after an overnight fast. Weight and height were measured, followed by a detailed medical history and physical examination conducted by a board-certified pediatric endocrinologist. Physical examinations included assessment of Tanner stage based on breast stage and pubic hair development in girls [13] and genitalia development in boys [14].

Inpatient Visit. Children were admitted to the General Clinical Research Center in the early afternoon and then underwent tests for bone mass and body composition with the use of dual-energy X-ray absorptiometry. Abdominal fat distribution was measured by MRI.

Detailed Methodologies

Weight, Height and BMI. Height (using a wall-mounted stadiometer) and weight (using a balance beam medical scale) were recorded at each visit to the nearest 0.1 cm and 0.1 kg, respectively, and the average of the 2 measurements was used for analysis. BMI, BMI percentiles and z-scores for age and sex were determined based upon established CDC normative curves using Epi-Info 2000, version 1.1 (CDC, Atlanta, Ga., USA).

Serum Leptin, Estradiol and Testosterone. Fasting plasma leptin was measured by radioimmunoassay kits (Linco Research, St. Charles, Mo., USA) with an intra-assay coefficient of variation (CV) of 3.9% and an interassay CV of 8.5%. Serum estradiol was measured by double-antibody radioimmunoassay (Diagnostics Products Corp., Los Angeles, Calif., USA; 200 μ l of sera per test; sensitivity: 2.15 pg/ml; intra-assay CV 5.3% and interassay CV 6.0%). Testosterone was measured by coated-tube radioimmunoassay (Diagnostics Systems Laboratories, Webster, Tex., USA; 50 μ l of sera per test; sensitivity: 12 ng/dl; intra-assay CV 7.7% and interassay CV 8.2%).

BMC, Bone Area and Body Composition. A whole-body dual-energy X-ray absorptiometry scan was performed to determine total-body BMC (in grams), bone area (BA; in square centimeters), fat mass and lean mass (i.e. lean without BMC) using a Hologic QDR 4500 W (Hologic Inc., Bedford, Mass., USA). The whole-body scan requires the subject to be placed supine with the

arms and legs positioned according to the manufacturer's specifications. Quality control was performed daily using a phantom. The precision error (CV for repeated measurements) was <1% for the whole body.

Fat Distribution. SAAT and IAAT were measured by MRI at the Los Angeles County/USC Imaging Science Center. Single-slice T1-weighted scans were taken at the umbilicus with participants in the supine position. Scans lasted approximately 2 min. A General Electric 1.5 Signa LX-Echospeed device with a General Electric 1.5-tesla magnet and body coil were used (General Electric, Waukesha, Wisc., USA). Field-of-view ranges were between 432 and 500 mm, and the image matrix was 256 × 256. Slice thickness was 10 mm, repeat-time ranges were between 516 and 533 ms and echo time ranged from 17 to 20 ms. A manual delineation of the subcutaneous (between the skin and abdominal wall muscles) and the abdominal (within the abdominal wall muscles) areas for SAAT and IAAT, respectively, was made with a light pen on the computer screen by a trained radiation imaging technologist. Data were integrated automatically using imbedded software provided by the manufacturer. A single radiologist reviewed all images after processing. The CVs for SAAT and IAAT from single scans performed at this imaging center are 0.9 and 2.3%, respectively [15].

Statistical Analysis

All analyses were performed using SPSS, version 14.0 (SPSS Inc., Chicago, Ill., USA), with a type I error set at $p < 0.05$. Independent-sample *t* tests were performed to determine whether there were sex differences in participant characteristics. Pearson and partial correlations were performed to determine the unadjusted and adjusted relationships between BMC and BA and the independent variables. BMC was not normally distributed and was log transformed for the multiple linear regression analyses. Stepwise multiple linear regression analysis was used to identify the significant covariates of log BMC.

Results

Means and standard deviations of participant characteristics are shown in table 1. The youngest child was 8 years old; the oldest was 14. The median BMI in girls was 27.6, with a range of 18–44. The median BMI in boys was 27.3, with a range of 18–47. Tanner stage, percentage fat, serum leptin and estradiol levels of girls were significantly greater than those of boys; the girls had significantly lower testosterone levels than boys. There were no other significant differences between girls and boys.

The strongest covariates of BMC and BA were age, Tanner stage and weight, with *r* values in the range of 0.83–0.90 in girls and 0.64–0.82 in boys (table 2). The associations between SAAT and BMC or BA as well as between SAAT + IAAT and BMC or BA were negative, with stronger *r* values observed in girls compared with boys. Because sex hormones are confounders of the association

Table 1. Descriptive characteristics of our study population

Variable	Girls (n = 111)	Boys (n = 145)
Age, years	11.0 ± 1.8	11.1 ± 1.6
Tanner stage	3 ± 1	2 ± 1*
Tanner 1	26 (24)	79 (54)
Tanner 2	27 (24)	46 (32)
Tanner 3	15 (14)	7 (5)
Tanner 4	27 (24)	7 (5)
Tanner 5	16 (14)	6 (4)
Weight, kg	63.4 ± 20.3	65.1 ± 20.0
Height, cm	147.9 ± 11.6	149.8 ± 11.1
BMI	28.3 ± 5.5	28.4 ± 5.7
BMI percentile	96.0 ± 2.2	95.9 ± 2.5
BMI z-score	2.1 ± 0.4	2.0 ± 0.5
Fat mass, kg	25.5 ± 10.3	24.6 ± 10.7
Percentage fat	39.8 ± 5.5	37.5 ± 7.0*
Lean mass, kg	35.6 ± 10.1	37.9 ± 10.1
Leptin, µg/l ^a	30.1 ± 13.5	22.5 ± 11.1*
Estradiol, pg/ml ^b	23.0 ± 33.7	4.9 ± 4.0*
Testosterone, ng/dl ^c	29.2 ± 13.6	164.0 ± 166.2*
SAAT, cm ²	344.0 ± 137.0	330.9 ± 156.8
IAAT, cm ²	48.8 ± 20.2	47.9 ± 23.0
SAAT + IAAT, cm ²	392.8 ± 151.1	378.9 ± 171.0
Total-body BMC, g	1,512.2 ± 452.1	1,563.0 ± 420.3
Total-body BA, cm ²	1,608.9 ± 308.1	1,676.3 ± 291.1

Figures in parentheses represent percentages. * Significantly different to girls.

^a Leptin was measured in 212 subjects (92 girls, 120 boys).

^b Estradiol was measured in 73 subjects (48 girls, 25 boys).

^c Testosterone was measured in 111 subjects (49 girls, 62 boys).

between leptin and bone mass, these analyses were additionally adjusted for estradiol in girls and for testosterone in boys.

A total of 90% of the variance in BMC in girls was explained by weight (80%), Tanner stage (6%), age (2%) and leptin (2%). In boys, a total of 88% of the variance in BMC was explained by weight (67%), Tanner stage (1%), age (2%) and leptin (18%) (table 3).

Discussion

Our findings suggest that total abdominal adipose tissue is inversely correlated with BMC in Latino children. SAAT has a stronger inverse association with BMC and BA compared to IAAT. While IAAT was also independently associated with BMC and BA in girls, these

Table 2. Correlations with BMC and BA

Variables	BMC	BA
Age		
Girls	0.83**	0.83**
Boys	0.70**	0.72**
Tanner stage		
Girls	0.84**	0.83**
Boys	0.64**	0.58**
Weight		
Girls	0.90**	0.92**
Boys	0.82**	0.89**
BMI		
Girls	0.73**	0.76**
Boys	0.59**	0.66**
Fat mass		
Girls	0.77**	0.80**
Boys	0.58**	0.68**
Lean mass		
Girls	0.76**	0.78**
Boys	0.70**	0.72**
SAAT ^a		
Girls	-0.38*	-0.43**
Boys	-0.26*	-0.25*
IAAT ^b		
Girls	-0.32*	-0.36*
Boys	-0.12	-0.11
SAAT + IAAT ^c		
Girls	-0.47**	-0.52**
Boys	-0.28*	-0.27*
Leptin		
Girls (n = 48) ^d	-0.39*	-0.44*
Boys (n = 62) ^e	-0.38*	-0.37*

* p < 0.05; ** p < 0.0001.

^a Adjusted for age, Tanner stage, weight or fat mass and IAAT.

^b Adjusted for age, Tanner stage, weight or fat mass and SAAT.

^c Adjusted for age, Tanner stage and weight or fat mass.

^d Additionally adjusted for estradiol and fat mass (instead of weight).

^e Additionally adjusted for testosterone and fat mass (instead of weight).

observations were not significant in boys. The sex difference in the inverse correlation between IAAT and BMC was unexpected. We found that girls were more mature, had a higher percentage of body fat, higher serum leptin and estradiol levels but lower testosterone compared to boys. Since the partial correlations between IAAT and BMC were adjusted for age, Tanner stage, weight and SAAT, the sex difference in the asso-

Table 3. Multiple linear regression model for log BMC

	Girls (n = 92)		Boys (n = 120)	
	b ± SE	partial R ²	b ± SE	partial R ²
Intercept	6.16 ± 0.10		6.41 ± 0.07	
Age	0.05 ± 0.02*	0.02	0.02 ± 0.01*	0.02
Tanner stage	0.04 ± 0.02*	0.06	0.03 ± 0.01*	0.01
Weight	0.01 ± 0.00**	0.80	0.01 ± 0.00**	0.67
SAAT	NS		NS	
IAAT	NS		NS	
Leptin	-0.005 ± 0.00**	0.02	-0.008 ± 0.00**	0.18
Total R ²	0.90		0.88	

b = Multiple regression unstandardized coefficient; SE = standard error; NS = not significant. * p < 0.05; ** p < 0.0001.

ciation between IAAT and BMC may be explained by endocrine differences.

In the entire cohort, we observed a positive correlation between IAAT and leptin, a cytokine-like protein produced by the *ob* gene thought to be integral in regulating appetite and energy expenditure [16]. Since girls were more mature than boys, leptin and Tanner stage were positively correlated in girls but negatively correlated in boys, and leptin and BMC were inversely correlated, it is plausible to conclude that the adverse effect of IAAT on BMC in girls may be explained, at least partially, by leptin.

Besides the fact that Tanner stage and leptin were inversely related in boys, other explanations for the lack of significant findings with respect to IAAT and BMC in boys include an inverse association between testosterone concentrations and leptin. Since boys had higher levels of testosterone than girls, these inverse associations are additional support. The possible links between leptin and developmental changes in children have been reported [17]. Previous researchers [18] have indicated that leptin may play a role in pubertal initiation. However, the associations between leptin and sex hormones need to be validated by other researchers, examined in different ethnic populations of adolescents and explored further in longitudinal studies of this cohort.

Previous research on the role of leptin in bone mineral density and BMC has been inconclusive, partly due to the fact that confounders of the relationships have not always been taken into account. In children, Matkovic et al. [19] found an inverse relationship between leptin and

total-body BA in pubertal girls. In contrast, after adjusting for age, Tanner stage, BMI and fat mass, Huang et al. [20] did not find correlations between leptin and BMC in 105 Taiwanese adolescents; however, they did not adjust for lean mass, estradiol or testosterone. Similarly, Roemich et al. [21] concluded that leptin did not contribute to the prediction of BMC in 59 Caucasian children, after accounting for age, fat mass, fat-free mass and estradiol, but they did not include Tanner stage in their models. On the other hand, Garnett et al. [22] concluded that leptin had a positive effect on spine bone mineral density of 255 children, but no such effect was observed on BMC, which is a more accurate and reliable measure of bone in children [23]. The current investigation is the first to examine the independent effect of leptin on BMC while adjusting for important confounders in pre- and postpubertal Latino children.

The inconclusive effect of leptin on BMC may also be explained by its dual role and alternative pathways, one involving a direct stimulatory effect on bone formation and another involving an indirect effect through the central nervous system that suppresses bone formation [24]. Our results are consistent with in vitro experiments and animal studies that show that leptin has an inhibitory effect on new bone formation and that the effect does not

seem to be mediated through endocrine or paracrine action, but through the effect of leptin on the central nervous system [25]. Studies have shown specific groups of neurons in the hypothalamus which are involved in the antiosteogenic function of leptin, and these neurons are different from the ones that regulate energy metabolism [25].

The interrelationships between abdominal adipose tissue, leptin and puberty will remain speculative until they are assessed in longitudinal studies of children. Until then, the detrimental effects of central adiposity on the skeletal system of adolescents should not be ignored. As the obesity epidemic in children continues to be a health crisis, whether visceral and subcutaneous fat and total central adiposity are risk factors for inadequate acquisition of BMC during growth and for osteopenia and fracture risk later in life should be of paramount concern.

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