

Low HDL Cholesterol May Lead to Disruption Of Bone (re) Modeling in Obese Early Pubertal Girls

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ABSTRACT

AIM: Cardiovascular risk factors and compromised bone phenotype are apparent in obese children. Identification of cardiovascular-bone linking factors is warranted. The objective is to determine the association between circulating factors of lipid metabolism and bone parameters in obese early-pubertal females.

MATERIALS AND METHODS: 43 peri-pubertal obese girls aged 7-11 years who underwent dual-energy x-ray absorptiometry (DXA) to measure whole body bone mineral content (BMC) and peripheral quantitative computed tomography (pQCT) to measure bone parameters were included. Lipids (total cholesterol, TC; high-density lipoprotein cholesterol, HDL; low-density lipoprotein cholesterol, LDL; triglyceride, TG), bone turnover markers (carboxy-terminal collagen crosslinks, CTX; type 1 procollagen N-terminal propeptide, P1NP), and inflammatory cytokines (tumor necrosis factor alpha, TNF α ; interleukin-6, IL-6) were obtained from venipuncture.

RESULTS: HDL was positively associated with P1NP ($p < 0.01$) and total bone area at 66% radial length, yet negatively associated with total bone density ($p \leq 0.05$). Stratification by HDL level revealed

significant differences in total bone area at 66% radial length between normal- and low-HDL groups. TNF α and IL-6 contributed to the observed differences ($p \leq 0.05$).

CONCLUSION: The findings herein indicate a link between lipid and inflammatory markers and bone parameters in overweight/obese girls, which may partially explain the increased risk of fracture in this population.

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Key words: Bone (re)modeling; Cytokines; HDL cholesterol; Obesity; Puberty

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INTRODUCTION

The link between osteoporosis and heart disease is fairly well recognized^[1,2]. Additionally, it is accepted that many cardiovascular risks factors (e.g. altered lipid profile, inflammation) are established in childhood, particularly as a consequence of excess adiposity^[3,4]. Recently, and contrary to the reportedly ascribed benefits of obesity to bone, these risk factors have been found to induce more adverse skeletal phenotypes, manifesting as increased fracture risk, osteoporosis or osteoarthritis in adults^[5,6]. Obese children, despite greater bone density relative to their normal weight peers, experience increased fracture prevalence suggesting potential obesity-induced alterations in bone^[7,8].

Lipid metabolism, integral in bone development, may play a role in obtainment and maintenance of components which effect skeletal integrity, particularly during growth and development. Studies have established a mechanistic link between lipid metabolism and a number of bone-related processes that affect mineral deposition and bone resorption^[9-11]. At the cellular level, a dysregulated lipid

profile (e.g. elevated low-density lipoprotein [LDL], decreased high-density lipoprotein [HDL]) alters bone (re)modeling processes via influence on mesenchymal stem cell (MSC) lineage within the marrow compartment promoting increased adipogenesis at the expense of osteoblastogenesis. Such alterations in (re)modeling have been shown to adversely affect structural properties of bone even in the absence of decrement in bone mass^[12,13]. Both elevated levels of LDL and decreased levels of HDL have been correlated with osteoporosis, increased bone marrow fat and greater fracture risk in women^[14,15]. Additionally, lipids are key signaling molecules that regulate the inflammatory cascade and evidence suggests that inflammatory cytokines such as tumor necrosis factor alpha (TNF α) and interleukin 6 (IL-6) may disrupt skeletal (re)modeling^[16,17]. These consequential skeletal effects correlated with altered lipid metabolism and inflammation are particularly relevant during puberty when a large portion of strength-structural properties are established.

The foundation for long term bone health is established during puberty, a critical period for the attainment of strength-structural properties of bone through (re)modeling processes. Accordingly, actions to maintain normal physiological properties (i.e. lipid profile, bone modeling) during the pubertal transition are vital to optimize growth. This is of particular relevance among overweight/obese adolescents, who reportedly experience perturbations in lipid metabolism and heightened inflammation. Although bone metabolism, the inflammatory cascade, and lipid metabolism are linked, few studies have been conducted which investigate these interrelationships and none during growth and development. As such, the objective of this study is to determine the association between circulating factors relating to lipid metabolism, inflammation, and markers of bone turnover, as well as evaluate the relationship with structural aspects of bone in obese early-pubertal females.

METHODS

Participants included 43 peri-pubertal (Tanner stages II-IV) overweight/obese girls aged 7-11 years enrolled in a dietary intervention aimed at improving body composition and metabolic health. Overweight and obesity was defined as $\geq 85^{\text{th}}$ and $> 95^{\text{th}}$ sex- and age-specific BMI percentile, respectively. The data presented herein represent a cross sectional assessment of anthropometric and biologic samples obtained at baseline. The protocol was approved by the Institutional Review Board for human subjects at the University of Alabama at Birmingham (UAB). All measurements were obtained at UAB between 2011 and 2013.

Protocol

The details of the dietary intervention have been previously described^[18]. Briefly, participants attended two visits at baseline. The first visit included a physical examination by the study pediatrician, questionnaires on typical diet and regular physical activity, and measures of body composition. At the second visit, participants arrived in the morning in the fasted state for metabolic testing.

Anthropometrics

A registered dietitian obtained heights and weights for all participants. Participants were weighed (Scale-Tronix 6702W; Scale-Tronix, Carole Stream, IL, USA) to the nearest 0.1 kg in minimal clothing without shoes. Height was also recorded without shoes using a digital stadiometer (Heightronic 235; Measurement Concepts, Snoqualmie, WA, USA). BMI percentile and overweight/obesity status was calculated using sex- and age-specific CDC growth charts based on

these measurements (<http://apps.nccd.cdc.gov/dnpabmi/>).

Body Composition

Whole body bone mineral content (BMC), a quantitative assessment of bone health, was obtained by a dual energy x-ray absorptiometry (DXA) scan using a GE Lunar Prodigy densitometer (GE LUNAR Radiation Corp., Madison, WI). Participants were scanned in light clothing, while lying flat on their backs with arms at their sides. DXA scans were performed and analyzed using pediatric software (encore 2002 Version 6.10.029). In our laboratory, the coefficient of variation (CV) for repeated measures of total body fat mass is 6.55%. Additionally skeletal components were assessed at the radius via a peripheral quantitative computed tomography (pQCT) scan using the Stratec 3000XCT (White Plains, NY). The radial length was measured from the radius head to the styloid process (mm). One slice was taken at 4% (representing primarily trabecular bone) and 66% (representing primarily cortical bone) of the total length defined by a region of interest and then analyzed using the macro software to quantify total bone area, total bone density, trabecular bone area, trabecular bone density, cortical bone area, cortical bone density, and stress strain index.

Assay of Metabolites

Lipid profile, inflammatory markers and bone turnover markers were assessed in the fasted state. Triglycerides (TG) were measured with the glyceryl phosphate method. HDL was analyzed using a two-reagent system involving stabilization of LDL, very low-density lipoprotein cholesterol (VLDL), and chylomicrons using cyclodextrin and dextrin sulfate, and subsequent enzymatic-colorimetric detection of HDL. Carboxy-terminal collagen crosslinks (CTX; intra-assay CV 9.14, inter-assay CV 6.29, sensitivity 0.178 ng/ml), a marker of bone resorption, and serum type 1 n-terminal procollagen (PINP; intra-assay CV, 3.06, inter-assay CV 3.98, sensitivity 5.6 ug/L), a marker of bone deposition, were measured by RIA and ELIZA (ImmunoDiagnosticSystems, Fountain Hills, AZ), respectively. Inflammatory markers, tumor necrosis factor alpha (TNF α ; inter-assay CV 5.47, intra-assay CV 7.61, sensitivity 0.507 pg/mL) and interleukin 6 (IL-6; inter-assay CV 9.72, intra-assay CV 6.68, sensitivity 0.25 pg/mL), were measured by chemiluminescence (MesoScale Discovery, Gaithersburg, MD).

Statistical Analysis

Participants were grouped by HDL status according to American Heart Association guidelines for children^[4] such that those that presented with an acceptable level (>35 mg/dL) were placed in the "Normal" Group and those that presented with a borderline to low level (≤ 35 mg/dL) were placed in the "Low" Group. Descriptive statistics (mean \pm standard error) were evaluated to characterize the overall sample as well as by HDL group. Data transformations were made as necessary to meet statistical assumptions. Bivariate Pearson correlations were used to examine the relationships among BMC, total bone area and density, cortical bone area and density, trabecular bone area and density, and stress strain index, TC, LDL, HDL, TG, PINP, CTX, TNF α and IL-6. Analysis of variance was used to examine the independent associations between bone parameters, lipids and markers of inflammation adjusting for race, pubertal status, and BMI percentile with adjusted means presented (LSMEANS). Variable inclusion in the model was based upon priori associations identified in bivariate correlations. All statistical analyses were performed using SAS software (version 9.2, SAS Institute Inc., Cary NC) and a two-sided P-value of 0.05 was considered statistically significant.

RESULTS

Descriptive statistics for the total sample and by HDL group are listed in table 1. Participants (51% African American, 40% White, 9% other) were on average 9.7 years of age and weighed 135.4 pounds with an average BMI greater than the 97th percentile. When comparing between groups, the normal-HDL group had significantly higher P1NP levels than the low-HDL group; however, no other measures were significantly different between groups.

Bivariate correlations (Table 2) revealed positive correlations

Table 1 Descriptive characteristics for the total sample and by HDL group.

	Total (n=43)		Normal-HDL Group (n=34)		Low-HDL Group (n=9)	
	Mean	SE	Mean	SE	Mean	SE
Anthropometrics						
Age (yrs)	9.7	0.2	9.6	0.2	9.8	0.4
Tanner	2.5	0.1	2.6	0.1	2.1	0.5
BMI (kg/m ²)	29.8	1.1	30.6	1.2	26.7	1.6
Height (in.)	57.3	0.6	57.4	0.7	57.1	0.9
Weight (lbs)	135.4	4.4	135.8	4.9	133.9	10.0
Total Fat (kg)	26.4	1.0	26.4	1.1	26.6	2.5
Lipid Parameters						
TC (mg/dL)	148.6	3.9	152.1	4.5	135.3	7.0
TG (mg/dL)	76.9	4.6	75.7	4.7	81.2	13.0
LDL (mg/dL)	89.6	3.4	90.0	4.0	88.2	6.1
HDL (mg/dL)*	43.6	1.6	47.0	1.6	30.9	1.2
Markers of Inflammation						
TNFα (pg/mL)	4.5	0.3	4.5	0.4	4.5	0.7
IL-6 (pg/mL)	1.7	0.2	1.5	0.2	3.1	1.9
Bone Turnover Markers						
CTX (ng/mL)	2.0	0.1	2.0	0.1	1.8	0.2
P1NP (ug/L)*	672.0	38.2	715.0	39.6	448.3	42.9
Bone Quantity						
BMC (g)	1627.5	49.9	1625.9	55.7	1633.6	119.3

* indicates significant difference between groups ($p \leq 0.05$). Abbreviations: yrs: years; BMI: body mass index; kg: kilograms; in: inches; lbs: pounds; BMC: whole body bone mineral content; CTX: carboxy-terminal collagen crosslinks; P1NP: procollagen type 1 amino-terminal propeptide; TC: total cholesterol; TG: triglycerides; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TNFα: tumor necrosis factor alpha; IL-6: interleukin 6.

Table 2 Bivariate correlation matrix.

	TC	TG	LDL	HDL	TNFα	IL-6	CTX	P1NP	BMC	4%		66%	
										Total Area	Trabecular Area	Total Area	Total Density
TC													
TG	0.12												
LDL	0.90	0.01											
HDL	0.44	-0.26	0.10										
TNFα	0.07	0.13	0.09	-0.05									
IL-6	0.35	0.26	0.45	-0.15	-0.12								
CTX	-0.09	0.13	-0.06	-0.14	0.56	-0.16							
P1NP	0.05	-0.17	-0.10	0.45	-0.14	-0.40	0.49						
BMC	-0.17	0.01	-0.12	-0.08	0.27	0.03	0.49	0.27					
4%													
Total Area	-0.39	0.08	-0.33	-0.30	0.20	-0.08	0.34	0.22	0.64				
Trabecular Area	-0.39	0.08	-0.33	-0.30	0.12	-0.08	0.34	0.22	0.64	1.00			
66%													
Total Area	0.14	-0.15	0.01	0.43	0.59	-0.12	0.19	0.30	0.19	-0.07	-0.07		
Total Density	-0.11	0.08	0.03	-0.38	-0.52	0.43	-0.08	-0.31	0.28	0.29	0.29	-0.62	

Bold indicates significant correlations between variables ($p \leq 0.05$). Abbreviations: TC: total cholesterol; TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; TNFα: tumor necrosis factor alpha; IL-6: interleukin 6; CTX: carboxy-terminal collagen crosslinks; P1NP: procollagen type 1 amino-terminal propeptide; BMC: whole body bone mineral content.

Table 4 The differences in qualitative and quantitative bone parameters by HDL group.

Independent Variable	TC				TG				LDL				HDL			
	Normal		Low		Normal		Low		Normal		Low		Normal		Low	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Bone Parameters																
4% of Radial Length																
Total Area	228.8	15.0	238.8	31.2	223.1	16.5	261.0	33.5	223.9	15.4	258.2	31.3	235.2	16.3	214.2	37.3
Trabecular Area	102.8	6.7	107.4	14.0	100.3	7.4	117.3	15.1	100.6	6.9	116.1	14.1	105.7	7.3	96.3	16.7
66% of Radial Length																
Total Area	210.5	28.7	199.6	14.1	201.6	14.0	203.1	28.1	201.3	14.1	204.3	28.2	185.8†	12.8	262.5*	29.4
Total Density	537.5	21.2	603.0	43.2	535.8	21.0	609.4	42.1	536.1	21.1	608.1	42.1	550.7	21.2	553.3	48.5

Model adjusted for lipid markers, race, pubertal status, and BMI. * indicates significant difference between groups when adjusting for specific lipid marker ($p \leq 0.05$). Abbreviations: TC=total cholesterol, TG=triglycerides, LDL=low-density lipoprotein, HDL=high-density lipoprotein

between CTX and BMC ($p < 0.01$), P1NP ($p < 0.01$) and TNFα ($p = 0.01$). A positive correlation between HDL and P1NP ($p < 0.01$), and LDL and IL-6 ($p = 0.05$) was observed. At 4% of radial length, total bone area and trabecular bone area were negatively correlated with TC ($p = 0.01$, $p = 0.01$, respectively), LDL ($p = 0.05$, $p = 0.05$, respectively) and HDL ($p = 0.03$, $p = 0.03$, respectively). At 66% of radial length, HDL was positively correlated with total bone area ($p < 0.01$), yet negatively correlated with total bone density ($p = 0.01$). Additionally, TNFα was positively correlated with total bone area ($p = 0.01$) and negatively correlated with total bone density ($p = 0.03$).

Table 3 and table 4 illustrate the differences in bone parameters by HDL group when adjusting for lipids and markers of inflammation. While no differences were observed in models that only adjusted for BMI, pubertal status and race, total bone area at 66% of radial length was significantly different between the normal-HDL group and the low-HDL group when TNFα, IL-6, or HDL were independently included in the model ($p \leq 0.05$ for all). Additionally, total bone density at 66% of radial length was also significantly different between groups with TNFα or IL-6 independently contributing to this difference ($p < 0.05$ for both). No statistically significant differences between groups were observed when analyzing bone measures at 4% of radial length.

Table 3 The difference in qualitative and quantitative bone parameters by HDL group.

Independent Variable	TNFα				IL-6					
	HDL Group		Normal		Low		Normal		Low	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
4% of Radial Length										
Total Area	210.2	24.1	227.3	75.5	210.8	25.2	223.5	88.5		
Trabecular Area	94.5	10.8	102.2	33.9	94.7	11.3	100.4	39.8		
66% of Radial Length										
Total Area	219.8†	13.0	105.5†	40.7	224.1†	18.0	75.4*	63.1		
Total Density	488.4†	15.9	701.0†	49.8	493.1†	18.2	668.2*	63.3		

Model adjusted for inflammatory markers (TNFα and IL-6), race, pubertal status, and BMI. * indicates significant difference between groups when adjusting for specific markers of inflammation ($p \leq 0.05$). Abbreviations: TNFα: tumor necrosis factor alpha; IL-6: interleukin 6.

DISCUSSION

The presumption that higher BMC in obese children is beneficial has been guided by historical data in adults in which bone was characterized solely by two-dimensional measures^[19,20]. However, increasing reports of greater fracture incidence in obese children relative to leaner counterparts, despite greater BMC, challenges this assertion^[7,8]. Bone strength is ultimately determined by the spatial distribution of mineral (i.e. at periosteal and endosteal surfaces) rather than simple DXA-derived areal bone measures, and studies investigating bone properties should include such comprehensive evaluations. Moreover, as a highly vascularized endocrine organ, circulating factors that are modified by obesity and known to affect the strength-structural properties of bone may provide further insight. The findings reported herein indicate a positive association between bone formation marker, P1NP, and HDL, as well as contradicting associations between HDL and measures of radial bone area and density. Additionally, markers of inflammation and HDL partly accounted for differences between individuals with normal or low HDL in total bone area, trabecular bone area, and total bone density in the radius. These findings support a link between HDL, as well as inflammation and bone measures of non-weight bearing sites during early puberty.

Though the pathway is not fully understood, oxidized lipids, which may accompany a dyslipidemic profile, disrupt bone (re)modeling processes^[11,21]. While studies in humans are lacking, dyslipidemia has been shown to impair osteogenesis^[22,23] via lipid accumulation in the subendothelial matrix within osteons and subsequent lipid oxidation^[21]. Our findings of a negative association of TC and LDL with total and trabecular bone area at 4% of radial length align accordingly with previous reports^[14,24]. Dissimilar to that previously reported, HDL was also negatively associated with total and trabecular bone area at 4% of radial length. Speculatively, HDL may preserve skeletal aspects concordant with the (re)modeling of long bones during puberty in which there is a predictable decrease in shaft diameter during optimal bone growth^[25]. However, at 66% of radial length the relationship between HDL and total bone area differed, such that lower HDL was associated with lower total bone area. Additionally, lower HDL was also associated with greater total bone density at 66% of radial length. These findings show that HDL's role in bone growth may be skeletal-site dependent.

Moreover, dyslipidemia activates the inflammatory cascade characterized by increased circulating TNF α and IL-6^[26]. Specifically, TNF α and IL-6 have both been shown to directly and indirectly stimulate osteoclastogenesis and bone resorption^[26,27]. Additionally, TNF α induces apoptosis of chondrocytes, a primary constituent directing cartilaginous scaffolding in the bone matrix necessary for skeletal growth and elongation^[28]. Herein, those in the low-HDL group exhibited lower total bone area, yet greater total bone density when compared to the normal-HDL group. TNF α and IL-6 both independently contributed to these differences between groups. P1NP, a marker of bone formation, was also markedly reduced in the low-HDL group, although positively associated with HDL in the overall sample. Taken together, higher bone resorption with decreased collagen synthesis in the presence of decreased bone formation during a time of rapid growth and development may lead to altered skeletal expansion.

The strengths of this study include robust measures of comprehensively descriptive bone in which volumetric, as well as areal measures were able to be assessed. Despite this studies apparent strength, limitations were present. Measures from pQCT were

available at one time point. As such, only a cross-sectional look at bone parameters could be performed. Additionally, the samples size was minor, yet elucidation of potential relationships between lipid parameters and inflammatory markers with bone measures may help to guide future investigations.

CONCLUSION

Long-term skeletal health is established early in the life course and metabolic perturbations associated with excess adiposity during this time may disrupt (re)modeling processes leading to reduced bone integrity. The findings herein indicate a link between obesity-related alterations in lipid profile, as well as inflammation, and bone area and density in a non-weight bearing site, which may account for increased risk of fracture in this population. While these results are unable to distinguish the mechanistic underpinnings by which HDL and inflammatory markers alter skeletal development, it may guide the trajectory of future investigations focused on optimizing skeletal (re)modeling throughout the life course.

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