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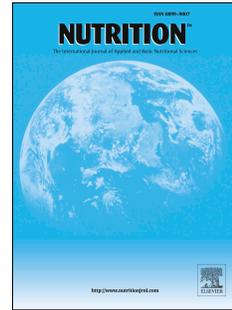


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TITLE PAGE

Title: Adiposity is associated with early reduction in bone mass in pediatric inflammatory bowel disease

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ABSTRACT

Background: The effect of adiposity on bone mass in the early phases of inflammatory bowel disease (IBD) in children and adolescents is unclear.

Aims: To determine the role of adiposity on bone mass in the first 3 years of diagnosis of IBD.

Hypothesis: Increased adiposity will be associated with increased bone mass in both the controls and IBD subjects.

Setting: University tertiary institution.

Methods: Height-adjusted bone mineral density (BMD) z-scores of 25 subjects, age 13.97 ± 2.70 y, diagnosed with IBD for < 4 years were compared to 24 controls, age 13.65 ± 2.60 y. Overweight was defined as BMI of $\geq 85^{\text{th}}$ but $< 95^{\text{th}}$ percentile, and obesity as BMI $\geq 95^{\text{th}}$ percentile. Severity of IBD was determined by the Pediatric Crohn's Disease Activity Index and Lichtiger Colitis Activity Index.

Results: Prior to stratification by BMI criterion, height-adjusted BMD z-scores were non-significantly lower in IBD subjects vs. controls for both the femoral neck (-0.8 ± 1.1 vs. -0.06 ± 1.1 , $p=0.070$) and lumbar vertebrae (-0.4 ± 1.2 vs. 0.2 ± 1.2 , $p=0.086$). Following stratification, height-adjusted BMD z-scores were significantly lower in the overweight/obese IBD subjects vs. overweight/obese controls for femoral neck (-0.9 ± 0.9 vs. 0.3 ± 1.3 , $p=0.032$); and non-significantly lower for the lumbar spine z-score (-0.4 ± 1.6 vs. 0.5 ± 1.3 , $p=0.197$). BMD z-score had no relationship with the duration of disease, steroid therapy, and the severity of disease.

Conclusion: Adiposity was associated with reduced bone mass in the early phases of IBD, but with increased bone mass in the controls.

INTRODUCTION

The term inflammatory bowel disease (IBD) refers to two diseases, Crohn's disease (CD) and ulcerative colitis (UC), which are characterized by chronic inflammation of the gastrointestinal tract, marked by recurrent periods of remission and exacerbation[1]. CD is characterized by discontinuous, transmural inflammation of the gastrointestinal tract with preferential involvement of the ileo-colonic segment, while UC is characterized by a more superficial, continuous inflammation that extends proximally from the rectum to variable areas of the large intestine[1].

There are two principal areas of controversy in the study of bone mineral density (BMD) in pediatric patients with IBD. The first is on the timing of the onset of decreases in bone mass in children and adolescents with IBD, and the second is on the role of adiposity on BMD in pediatric subjects with IBD. Currently, there is no consensus on the timing of onset bone loss in children and adolescents with IBD as some studies demonstrated early decreases in BMD [2-4], while others reported no change in BMD in the first few years of the disease[5].

Equally, there is no agreement on the role of adiposity on BMD in children and adolescents with IBD [6-9]. While several studies have reported a strong positive association between low BMI and decreased BMD in IBD [6, 7, 10], others found no such association [11, 12]. Conventionally, adiposity is believed to be beneficial to bone health given the positive effect of mechanical loading conferred by body weight on bone formation. However, this thinking has been challenged by recent studies that strongly suggest that fat accumulation is detrimental to bone mass [8, 9]. This novel thinking is predicated on several factors: both obesity and bone metabolism are interrelated as both osteoblasts and adipocytes are derived from a common mesenchymal stem cell[13]; agents that inhibit adipogenesis stimulate osteoblastic differentiation and bone formation, and vice versa [14-16]. Furthermore, obesity and osteoporosis are

associated with elevated oxidative stress and increased production of pro-inflammatory cytokines[17, 18] which promote osteoclastic activity leading to bone loss [19]. Finally, dietary factors such as high fat intake are believed to interfere with intestinal calcium absorption and therefore decrease calcium availability for bone formation.

One of the principal reasons for the lack of consensus on BMD status in the early phase of IBD in children and adolescents is because dual-X ray absorptiometry (DXA)-derived BMD is subject to potential misinterpretation as these measurements are influenced by body size[20]. Therefore bone density evaluation in IBD, where short stature is commonly seen, should not only be matched for age and gender, as this would over-estimate the proportion with spuriously low BMD, but should be further adjusted for size by calculating the height-adjusted BMD z-score[21-23].

The primary aim of this study was to determine the role of adiposity on bone mass in the first 3 years of diagnosis of IBD by comparing the BMD z-scores of IBD subjects to controls after stratifying by BMI criterion. The secondary aim was to determine the status of bone mass in the first 3 years of the diagnosis of IBD. The primary hypothesis is that increased adiposity will be associated with increased bone mass in both the controls and IBD subjects, while the secondary hypothesis is that bone mass will be lower in the first 3 years of IBD compared to controls.

SUBJECTS AND METHODS

Ethics Statement

The study protocol was approved by the University of Massachusetts Institutional Review Board (IRB). Written informed consent was obtained from parents, caretakers, or guardians on behalf of minors/children in the control group, who were originally enrolled in study NCT00756899, entitled, 'Vitamin D deficiency and low bone mineral content (BMC) in children', a cross-sectional case control

study that was designed to determine the association of vitamin D status and BMC in healthy children. A waiver of authorization and a Health Insurance Portability and Accountability Act (HIPAA) waiver were obtained from the University of Massachusetts IRB to allow the investigators to access the medical records of the participants in the study group, and those in the control group who were not part of study NCT00756899. A written consent was not obtained from subjects not enrolled in NCT00756899 because the review of medical charts involved no more than minimal risk to the patients, and it would be impossible to conduct the research without a waiver of authorization as some of the patients had either left the clinic or moved out of the state. The IRB approved the use of a waiver of authorization for these cases. All patient records and information were anonymized and de-identified prior to analysis.

Control subjects

As mentioned above, participants in the control group (n=24) were drawn from a study to evaluate the association of vitamin D status and bone mineral density (clinical trial identification number NCT00756899), and from the medical records of otherwise healthy pediatric patients of <18 years (y) who had BMD assessment as part of their clinical evaluation for bone complaints such as traumatic fracture while skiing. Subjects were excluded from the control group if they had any systemic illnesses such as diabetes mellitus, chronic renal disease, or known metabolic or genetic diseases resulting in obesity such as severe hypothyroidism, pseudohypoparathyroidism, or Cushing's disease. Methods used for exclusion included history, physical examination, and screening laboratory tests for fasting blood glucose, cortisol, urinalysis, comprehensive metabolic panel, serum creatinine, and thyroid function tests.

Study subjects

The study group consisted of 25 children and adolescents of ages 7-18 years who were diagnosed with either Crohn's disease (CD, n = 15) or ulcerative colitis (UC, n = 10). Subjects were included in the study group if their diagnosis of CD or UC was established by a combination of standard clinical, endoscopic, and radiographic criteria, as documented at the time of diagnosis [24], as well as the availability of BMD

data measured in the first 3 years following the diagnosis of IBD. The severity of IBD was quantified with the Pediatric Crohn's Disease Activity Index (PCDAI)[25] and Lichtiger Colitis Activity Index (LCAI)[26]. Corticosteroid exposure was summarized as cumulative cortisone dose received before the first DXA scan [27, 28]. Subjects were excluded from the study group if they had diseases of calcium and/or vitamin D metabolism. Three subjects were excluded based on the above criteria.

Study Methods

Anthropometry:

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain Ltd, Crymych, Dyfed, UK) that was calibrated daily. Weight was measured to the nearest 0.1 kg using an upright scale. BMI was derived using the formula $\text{weight}/\text{height}^2$ (kg/m^2), and expressed as z-score for age and gender based on National Center for Health Statistics (NCHS) data[29]. Anthropometric data were expressed as mean \pm SD.

Bone Mineral Density Analysis

Participants in the control and study group were evaluated for bone mineral density of their compact (femoral neck) and cancellous (lumbar spine) bones using a Hologic Discovery C, QDR Series DXA scanner (Bedford, MA). The software used for the interpretation of the scanned results was the Apex System Software Version 3.3. All BMD results were expressed as z-scores. Measurements were taken at both the femoral neck and lumbar spine (L1-L4) according to the manufacturer's specifications. The delineations for the regions of interest were done automatically with the integrated software. Height-adjusted BMD z-scores were determined using the Bone Mineral Density in Childhood z-score online calculator developed by Zemel et al[23] and located at <http://www.bmdcspublic.com/zscore.htm>.

Statistical Analyses

Statistical analyses were performed using the SPSS Predictive Analytics Software version 21 (IBM Corporation, Armonk, NY, USA). Normal probability plots were constructed with the variables of interest. No departure from normality was detected, allowing for the use of parametric tests, without the need for transformation. Means, standard deviations and percentages were calculated for descriptive summary statistics. Proportions were compared using Chi square tests. Overweight was defined as BMI of $\geq 85^{\text{th}}$ but $< 95^{\text{th}}$ percentile, while obesity was defined as a BMI of $> 95^{\text{th}}$ percentile for age and sex. Data were expressed as mean \pm standard deviation (SD).

RESULTS

Table 1 shows the mean (\pm SD) values for anthropometric, radiological, and therapeutic parameters. The mean duration of disease for the study patients was 1.33 ± 1.4 y, with a median duration of 0.55 years, and minimum of 0.06 year and maximum of 3.82 years. The IBD cohort was significantly shorter than the controls, and had a higher frequency for calcium and multivitamin supplementation. There were, however, no differences between the groups for age, gender, weight, BMI, race, and vitamin D supplementation.

Figure 1 depicts the comparison of the BMD z-scores for compact (femoral neck) and cancellous (lumbar vertebrae) bone for IBD and controls. Non-height-adjusted BMD z-scores were significantly lower in the IBD subjects vs. controls for both the femoral neck (-1.0 ± 1.3 vs. 0.04 ± 1.3 , $p = 0.007$) and lumbar vertebrae (-0.9 ± 1.3 vs. 0.3 ± 1.5 , $p = 0.006$). However, this effect was no longer significant after BMD z-scores were adjusted for height for femoral neck (-0.76 ± 1.14 vs. -0.06 ± 1.09 , $p = 0.070$) and lumbar vertebrae (-0.41 ± 1.16 vs. 0.19 ± 1.22 , $p = 0.086$) (Figure 1).

Figure 2 shows the comparison of femoral neck BMD z-scores between controls and subjects with IBD stratified by BMI into normal-weight (BMI $<85^{\text{th}}$ percentile) vs. overweight/obese (BMI $\geq 85^{\text{th}}$ percentile) subjects. Height-adjusted femoral neck BMD z-score was significantly lower in the overweight/obese IBD subjects compared to overweight/obese controls (-0.9 ± 0.9 vs. 0.3 ± 1.3 , $p=0.032$). In contrast, there was no difference in the height-adjusted femoral neck BMD z-score between the normal-weight controls vs. normal-weight IBD (-0.3 ± 1.0 vs. -0.7 ± 1.3 , $p = 0.310$) (Figure 2). Similarly, there was no significant difference in the height-adjusted femoral neck BMD z-score between the normal-weight vs. overweight/obese controls (-0.3 ± 0.1 vs. 0.3 ± 1.3 , $p = 0.249$) or normal-weight subjects with IBD vs. overweight/obese subjects with IBD (-0.7 ± 1.3 vs. $0.3 \pm -0.9 \pm 0.9$, $p = 0.600$).

Figure 3 shows the comparison of lumbar BMD z-scores between controls and subjects with IBD stratified by BMI. The non-height adjusted lumbar BMD z-score was significantly lower in the overweight/obese IBD subjects compared to the overweight/obese controls (-1.1 ± 1.1 vs. 0.6 ± 1.8 , $p = 0.035$). This effect was no longer significant after BMD z-scores were adjusted for height, as demonstrated by the lack of a difference in height-adjusted z-scores between the normal-weight controls vs. normal-weight IBD (-0.01 ± 1.2 vs. -0.4 ± 1.0 , $p=0.32$) or between the overweight/obese IBD subjects vs. overweight/obese controls (-0.4 ± 1.6 vs. 0.5 ± 1.3 , $p=0.197$). Equally, the height-adjusted lumbar spine BMD z-score did not differ between the normal-weight vs. overweight/obese controls (-0.01 ± 1.2 vs. 0.5 ± 1.3 $p = 0.306$), or between the normal-weight vs. overweight/obese subjects with IBD (-0.4 ± 1.0 vs. -0.4 ± 1.6 , $p = 0.991$).

Using an ANOVA to compare the BMI z-score between the IBD subjects and control groups showed a significant interaction between adiposity and gender ($p = 0.004$). However, the adiposity main effect was not significant for the IBD group ($p=0.85$). The imbalance appeared to occur in the female subjects in the control group who were much thinner than the male control subjects: -0.68 [-1.37 to 0.005] versus 1.38 [0.85 to 1.92]. This is in contrast to the comparable BMI z-score of the female IBD subjects of 0.19 [-0.54 to 0.92] to the male IBD subjects 0.40 [-0.11 to 0.90].

A comparison of BMD z-score between the IBD subtypes showed a lumbar z-score of -1.37 ± 1.22 for CD ($n=15$) versus -0.13 ± 1.20 for UC ($n=10$), ($p=0.02$). Femoral neck z-score was -1.39 ± 1.28 for CD versus -0.47 ± 1.12 for UC, ($p = 0.08$).

Regression analysis showed no relationship between the duration of disease and the BMD z-score of the lumbar spine ($r^2 = 0.30$, $\beta = -0.26$, $p = 0.29$) nor of the femoral neck z-score ($r^2 = 0.46$, $\beta = -0.25$, $p = 0.28$).

There was equally no relationship between the severity of disease and the BMD z-score of the lumbar spine ($r^2=0.45$, $\beta = 0.25$, $p = 0.44$) or the femoral neck ($r^2 = 0.39$, $\beta = 0.16$, $p = 0.67$).

Further analysis showed that the height-adjusted BMD z-score did not differ significantly by gender ($p=0.550$) between the controls and IBD subjects. There was also no difference in height-adjusted BMD z-score between the pubertal and post-pubertal subjects for either the lumbar spine ($p=0.978$) or the femoral neck ($p=0.170$) when IBD subjects were compared to controls.

Height-adjusted BMD z-score did not differ between patients who received steroid therapy and those who did not for femoral neck (-0.77 ± 1.3 vs. -0.54 ± 1.3 , $p = 0.66$), or for lumbar spine (-0.44 ± 1.6 vs. -0.40 ± 1.5 , $p = 1.0$). Cumulative prednisone dosages ranged between 150-4500 mg in subjects with IBD.

Two subjects in the control group had a history of occasional steroid therapy for the management of mild asthma while 4 subjects in the control groups received proton pump inhibitors for the treatment of gastro-esophageal reflux disease (Table 1).

DISCUSSION

This study found evidence that bone mass, as expressed by the height-adjusted **BMD z-scores** was **significantly lower for femoral neck** in the overweight/obese IBD subjects compared to overweight/obese controls. **Equally, lumbar spine z-score was lower in the overweight/obese IBD subjects compared to overweight/obese controls, though this did not reach statistical significance.** Among the IBD subtypes, both the femoral neck and lumbar spine z-scores were lower in the CD subjects compared to UC patients, though only the lumbar spine z-score reached statistical significance. This contrasting finding on femoral neck BMD suggests that while adiposity is associated with increased bone mass in control subjects, it has the opposite effect on BMD in patients with IBD.

While published studies have reported either a positive association[6, 7, 10], or the absence of an association[11, 12] between adiposity and bone mass in IBD; studies in healthy subjects have reported both positive[30] and negative [8, 9, 31] associations between adiposity and bone mass. Though the finding in the control subjects agrees with reports of positive relationship between adiposity and BMD in non-IBD subjects [30], no prior study, to our knowledge, has described a negative relationship between adiposity and BMD in IBD cohorts [9].

The mechanism of adiposity-related reduction in bone mass in IBD is multifactorial[28]; and there is no consensus on the etiology of the decreased bone mass in children and adolescents with IBD[3, 10, 32]. While the reduced bone mass is believed to arise from complex effects of fat mass on bone, it may also be partially attributed to factors such as inter-study differences in relation to gender, ethnicity, sample size, study design, methodology of analysis, and population structure[33]. However, this study reports no differences in height-adjusted femoral neck or lumbar spine BMD values when stratified by gender or pubertal status.

The reduced bone mass in IBD has also been linked to increased systemic inflammation[34] as some studies have reported that the monoclonal anti-TNF- α chimeric antibody, infliximab, has a beneficial effect on bone metabolism in children with IBD via the reduction of inflammatory cytokines and by improving serum vitamin D concentration through the healing of the vitamin D-absorptive surfaces[28, 35]. Our group[36] recently showed that seasons, skin pigmentation, and the degree of intestinal inflammation are the key non-dietary determinants of vitamin D status in children and adolescents with IBD[37]. In that study, subjects with IBD and elevated erythrocyte sedimentation rate (ESR) had significantly lower mean serum 25-hydroxyvitamin D [25(OH)D] concentration compared to controls. Furthermore, ESR showed a significant inverse relationship with serum 25(OH)D, while albumin and alanine transaminase (ALT) did not. These findings are consistent with reports that ESR is independently associated with 25(OH)D concentration[38, 39]. Therefore, increased inflammation in overweight/obese patients with IBD could lead to reduced bone mass by the direct effect of increased levels of inflammatory cytokines on bone metabolism through the promotion of osteoclastic activity [19], and indirectly by the reduction of serum vitamin D concentration through increased utilization of vitamin D's anti-inflammatory properties to counteract intestinal inflammation[40]. The decreased serum vitamin D concentration leads to reduced bone mass as vitamin D is the primary promoter of bone mineralization in humans.

Adiposity and IBD are associated with systemic inflammation[17, 28], however, the detection of a contrasting role for adiposity on bone mass in the controls compared to the IBD subjects suggests that the effect of obesity on bone mass may be related to the distinct pathogenesis of the adiposity. Specifically, exogenous or simple obesity as seen in the controls appears to increase bone mass, whereas pathological obesity, e.g., from steroid therapy or other causes, may result in decreased bone mass. It is known that certain therapeutic interventions may predispose affected individuals to both osteoporosis and obesity[41]. For example, osteoporosis and obesity are the two main side effects of treatment with gonadotropin-releasing hormone agonists, whose long-acting form is used for the management of central

precocious puberty, and its short-acting form is employed in the management of metastatic prostate cancer[42]. Equally, excessive use of corticosteroids has similar twin side effects of obesity and reduced bone mass. Twenty-eight percent of patients with IBD in our cohort received steroid therapy as compared to 8.3% in the controls. It is possible that these patients who received steroid therapy had evidence of subclinical hypercortisolism[31] with associated Cushingoid features of obesity. Such a therapy in this case is believed to suppress osteoblastic bone formation resulting in reduced bone mass[41], as studies have shown that agents that promote adipogenesis suppress osteoblastic differentiation and bone formation, and vice versa [14-16]. Furthermore, both adiposity and IBD are associated with vitamin D deficiency and their coexistence may further diminish bone mass in affected individuals. Therefore, the impact of adiposity on bone mass in IBD may be modified by the etiology of the obese phenotype. Studies that examined body fat in IBD demonstrated that though most patients with IBD have low or normal BMI, the ratio of their intra-abdominal fat to total abdominal fat far exceed that of non-IBD subjects[43, 44], such that some investigators use mesenteric white adipose tissue as a good indicator of active phase of Crohn's disease[45]. Therefore, it is likely that adiposity is underestimated in IBD patients.

In this study, corticosteroid therapy was not associated with significant decreased in BMD in this study. This could be due to the short duration of disease in our IBD cohort. However, there is no consensus on the effect of corticosteroid on bone mass in IBD. While one study reported that cumulative corticosteroid dose, height and BMI z-score were independent risk factors for low bone mass in adolescents with IBD[10], another study showed a decreased bone mass in steroid-naïve children with CD [46], and a third study reported no effect of glucocorticoids on BMD in Crohn's disease [2]. Thus, more studies are needed to determine the role of steroid therapy on bone mass in IBD [47].

The report of a lack of difference in height-adjusted BMD between normal-weight IBD vs normal-weight control children and adolescents subjects in this pediatric study differs from a case-

controlled study in adult subjects that found a higher prevalence of metabolic bone disease in patients with CD and BMI of $<18.5 \text{ kg/m}^2$ compared to controls[48]. In this adult study, factors associated with chronic active and long-lasting disease were associated with increased risk for metabolic bone disease. This is in contrast to the deleterious effect of adiposity on bone mass in the early phase of IBD in children and adolescents.

The limitations of this study include its cross-sectional design which limits causal inference on the effects of adiposity on bone mass in IBD. Second, we did not administer a food-recall questionnaire to accurately determine the vitamin D and calcium content of the subjects' diet as these may affect bone mass. The study's small sample size may have introduced type 2 error and precluded the detection of significant differences between the groups. We had no data on total body (minus head) BMD, as well as body composition analysis by DXA as such data would have served to corroborate our findings. Finally, our results were derived from a tertiary care center in a single state in the United States of America. Therefore, we are uncertain that our results are generalizable to other centers, states, or countries.

One of the strengths of this study is its case-controlled design which enabled us to compare parameters of interest in subjects with IBD to a control group. The control group was well-matched to the study group, thus limiting the effect of confounding variables. The study's core anthropometric and bone mineral density parameters were adjusted for covariates and expressed as z-scores. BMD z-scores were further adjusted for height to address the spuriously low BMD that would otherwise have resulted from the short stature associated with IBD.

CONCLUSION

Adiposity was associated with reduced bone mass in the early phases of IBD, but with increased bone mass in the controls. Further studies are needed to determine the effect of adiposity-associated inflammation on early decreases in bone mass in patients with IBD.

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CONFLICT OF INTEREST: The authors have no conflict of interest to declare.

ACCEPTED MANUSCRIPT

References

- [1] Day AS, Ledder O, Leach ST, Lemberg DA. Crohn's and colitis in children and adolescents. *World J Gastroenterol.* 2012;18:5862-9.
- [2] Dubner SE, Shults J, Baldassano RN, Zemel BS, Thayu M, Burnham JM, et al. Longitudinal assessment of bone density and structure in an incident cohort of children with Crohn's disease. *Gastroenterology.* 2009;136:123-30.
- [3] El-Matary W, Sikora S, Spady D. Bone mineral density, vitamin D, and disease activity in children newly diagnosed with inflammatory bowel disease. *Dig Dis Sci.* 2011;56:825-9.
- [4] Schmidt S, Mellstrom D, Norjavaara E, Sundh V, Saalman R. Longitudinal assessment of bone mineral density in children and adolescents with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* 2012;55:511-8.
- [5] Schoon EJ, Blok BM, Geerling BJ, Russel MG, Stockbrugger RW, Brummer RJ. Bone mineral density in patients with recently diagnosed inflammatory bowel disease. *Gastroenterology.* 2000;119:1203-8.
- [6] Ilich JZ, Skugor M, Hangartner T, Baoshe A, Matkovic V. Relation of nutrition, body composition and physical activity to skeletal development: a cross-sectional study in preadolescent females. *J Am Coll Nutr.* 1998;17:136-47.
- [7] Sabatier JP, Guaydier-Souquieres G, Benmalek A, Marcelli C. Evolution of lumbar bone mineral content during adolescence and adulthood: a longitudinal study in 395 healthy females 10-24 years of age and 206 premenopausal women. *Osteoporos Int.* 1999;9:476-82.
- [8] Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. *J Pediatr.* 2001;139:509-15.
- [9] Janicka A, Wren TA, Sanchez MM, Dorey F, Kim PS, Mittelman SD, et al. Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab.* 2007;92:143-7.
- [10] Lopes LH, Sdepanian VL, Szejnfeld VL, de Moraes MB, Fagundes-Neto U. Risk factors for low bone mineral density in children and adolescents with inflammatory bowel disease. *Dig Dis Sci.* 2008;53:2746-53.
- [11] Semeao EJ, Jawad AF, Stouffer NO, Zemel BS, Piccoli DA, Stallings VA. Risk factors for low bone mineral density in children and young adults with Crohn's disease. *J Pediatr.* 1999;135:593-600.
- [12] Abraham BP, Prasad P, Malaty HM. Vitamin D deficiency and corticosteroid use are risk factors for low bone mineral density in inflammatory bowel disease patients. *Dig Dis Sci.* 2014;59:1878-84.
- [13] Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. *Physiol Rev.* 1998;78:783-809.
- [14] Gimble JM, Robinson CE, Wu X, Kelly KA, Rodriguez BR, Kliewer SA, et al. Peroxisome proliferator-activated receptor-gamma activation by thiazolidinediones induces adipogenesis in bone marrow stromal cells. *Mol Pharmacol.* 1996;50:1087-94.
- [15] David V, Martin A, Lafage-Proust MH, Malaval L, Peyroche S, Jones DB, et al. Mechanical loading down-regulates peroxisome proliferator-activated receptor gamma in bone marrow stromal cells and favors osteoblastogenesis at the expense of adipogenesis. *Endocrinology.* 2007;148:2553-62.

- [16] Sen B, Xie Z, Case N, Ma M, Rubin C, Rubin J. Mechanical strain inhibits adipogenesis in mesenchymal stem cells by stimulating a durable beta-catenin signal. *Endocrinology*. 2008;149:6065-75.
- [17] Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest*. 2003;112:1785-8.
- [18] Mundy GR. Osteoporosis and inflammation. *Nutr Rev*. 2007;65:S147-51.
- [19] Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest*. 1995;95:2409-15.
- [20] Gordon CM, Bachrach LK, Carpenter TO, Crabtree N, El-Hajj Fuleihan G, Kutilek S, et al. Dual energy X-ray absorptiometry interpretation and reporting in children and adolescents: the 2007 ISCD Pediatric Official Positions. *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry*. 2008;11:43-58.
- [21] Herzog D, Bishop N, Glorieux F, Seidman EG. Interpretation of bone mineral density values in pediatric Crohn's disease. *Inflamm Bowel Dis*. 1998;4:261-7.
- [22] Wong SC, Catto-Smith AG, Zacharin M. Pathological fractures in paediatric patients with inflammatory bowel disease. *Eur J Pediatr*. 2014;173:141-51.
- [23] Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S, et al. Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. *J Clin Endocrinol Metab*. 2010;95:1265-73.
- [24] Ibd Working Group of the European Society for Paediatric Gastroenterology H, Nutrition. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis--the Porto criteria. *J Pediatr Gastroenterol Nutr*. 2005;41:1-7.
- [25] Hyams JS, Ferry GD, Mandel FS, Gryboski JD, Kibort PM, Kirschner BS, et al. Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr*. 1991;12:439-47.
- [26] Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med*. 1994;330:1841-5.
- [27] Ribeiro D, Zawadzinski S, Pittet LF, Chevalley T, Girardin E, Parvex P. Effect of glucocorticoids on growth and bone mineral density in children with nephrotic syndrome. *Eur J Pediatr*. 2015.
- [28] Pichler J, Hanslik A, Huber WD, Aufricht C, Bidmon-Fliegenschnee B. Paediatric patients with inflammatory bowel disease who received infliximab experienced improved growth and bone health. *Acta Paediatr*. 2014;103:e69-75.
- [29] Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, et al. 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Stat 11*. 2002;1-190.
- [30] De Laet C, Kanis JA, Oden A, Johanson H, Johnell O, Delmas P, et al. Body mass index as a predictor of fracture risk: a meta-analysis. *Osteoporos Int*. 2005;16:1330-8.
- [31] Greco EA, Fornari R, Rossi F, Santiemma V, Prossomariti G, Annoscia C, et al. Is obesity protective for osteoporosis? Evaluation of bone mineral density in individuals with high body mass index. *Int J Clin Pract*. 2010;64:817-20.
- [32] Paganelli M, Albanese C, Borrelli O, Civitelli F, Canitano N, Viola F, et al. Inflammation is the main determinant of low bone mineral density in pediatric inflammatory bowel disease. *Inflamm Bowel Dis*. 2007;13:416-23.
- [33] Wu H, Sandison G, Zhao L, Zhao Q, Shirato H, Jiang S. Correlation between parameters describing tumour motion and its location in the lungs. *Australas Phys Eng Sci Med*. 2007;30:341-4.

- [34] Veerappan SG, O'Morain CA, Daly JS, Ryan BM. Review article: the effects of antitumour necrosis factor-alpha on bone metabolism in inflammatory bowel disease. *Aliment Pharmacol Ther.* 2011;33:1261-72.
- [35] Malik S, Wong SC, Bishop J, Hassan K, McGrogan P, Ahmed SF, et al. Improvement in growth of children with Crohn disease following anti-TNF-alpha therapy can be independent of pubertal progress and glucocorticoid reduction. *J Pediatr Gastroenterol Nutr.* 2011;52:31-7.
- [36] Veit LE, Maranda L, Nwosu, B. U. The non-dietary determinants of vitamin D status in pediatric inflammatory bowel disease. *Nutrition.* 2015;in press.
- [37] Veit LE, Maranda L, Nwosu BU. The nondietary determinants of vitamin D status in pediatric inflammatory bowel disease. *Nutrition.* 2015;31:994-9.
- [38] Pappa HM, Gordon CM, Saslowsky TM, Zholudev A, Horr B, Shih MC, et al. Vitamin D status in children and young adults with inflammatory bowel disease. *Pediatrics.* 2006;118:1950-61.
- [39] Pappa HM, Langereis EJ, Grand RJ, Gordon CM. Prevalence and risk factors for hypovitaminosis D in young patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* 2011;53:361-4.
- [40] Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. *The Lancet Diabetes & endocrinology.* 2014;2:76-89.
- [41] Zhao LJ, Jiang H, Papasian CJ, Maulik D, Drees B, Hamilton J, et al. Correlation of obesity and osteoporosis: effect of fat mass on the determination of osteoporosis. *J Bone Miner Res.* 2008;23:17-29.
- [42] Smith MR. Osteoporosis and obesity in men receiving hormone therapy for prostate cancer. *J Urol.* 2004;172:S52-6; discussion S6-7.
- [43] Fink C, Karagiannides I, Bakirtzi K, Pothoulakis C. Adipose tissue and inflammatory bowel disease pathogenesis. *Inflamm Bowel Dis.* 2012;18:1550-7.
- [44] Desreumaux P, Ernst O, Geboes K, Gambiez L, Berrebi D, Muller-Alouf H, et al. Inflammatory alterations in mesenteric adipose tissue in Crohn's disease. *Gastroenterology.* 1999;117:73-81.
- [45] Drouet M, Dubuquoy L, Desreumaux P, Bertin B. Visceral fat and gut inflammation. *Nutrition.* 2012;28:113-7.
- [46] Harpavat M, Greenspan SL, O'Brien C, Chang CC, Bowen A, Keljo DJ. Altered bone mass in children at diagnosis of Crohn disease: a pilot study. *J Pediatr Gastroenterol Nutr.* 2005;40:295-300.
- [47] Walther F, Fusch C, Radke M, Beckert S, Findeisen A. Osteoporosis in pediatric patients suffering from chronic inflammatory bowel disease with and without steroid treatment. *J Pediatr Gastroenterol Nutr.* 2006;43:42-51.
- [48] Cravo M, Guerreiro CS, dos Santos PM, Brito M, Ferreira P, Fidalgo C, et al. Risk factors for metabolic bone disease in Crohn's disease patients. *Inflamm Bowel Dis.* 2010;16:2117-24.

TABLE**Tables and Table Headings**

Table 1: Characteristics of Subjects with Inflammatory Bowel Disease and Controls

Parameters	Controls	IBD	P value
Number of Subjects	24	25	
Age (years)	13.7 ± 2.6	14.0 ± 2.7	0.672
Gender: males %	(15/24), 62.5%	(17/25), 68.0%	0.458
Weight z-score	0.6 ± 1.5	0.023 ± 1.2	0.144
Height z-score	0.4 ± 1.0	-0.5 ± 1.3	0.004
Body mass index z-score	0.6 ± 1.4	0.3 ± 1.1	0.433
Lumbar (1-4) bone mineral content (BMC) (g/cm ²)	0.9 ± 0.2	0.8 ± 0.2	0.092
Non-height adjusted lumbar (L1-L4) vertebrae z-score	0.3 ± 1.5	-0.9 ± 1.3	0.006
Height-Adjusted lumbar (1-4) vertebrae z-score	0.2 ± 1.2	-0.4 ± 1.2	0.086
Femoral neck BMC (g/cm ²)	0.8 ± 0.1	0.7 ± 0.2	0.070
Non-height adjusted femoral neck z-score	0.04 ± 1.3	-1.0 ± 1.3	0.007
Height-Adjusted femoral neck z-score	-0.06 ± 1.1	-0.8 ± 1.1	0.070
Body mass index >85 th percentile	(9/24), 37.5%	(8/25), 32.0%	0.458
Race: non-Hispanic whites (%)	(21/24) 87.5%	(23/25), 92.0%	0.306
Steroid therapy (prednisone)	(2/24), 8.3%	(7/25), 28.0%	0.088
Multivitamin supplementation	0%	(12/25), 48.0%	<0.001
Vitamin D supplementation	(4/24), 16.7%	(9/25), 36.0%	0.134
Calcium supplementation	(2/24), 8.3%	(8/25), 32.0%	0.054
Proton pump inhibitor therapy	(4/24), 16.7%	(10/25), 40.0%	0.067
6-Mercaptopurine therapy	0%	(1/25), 4.0%	0.216
Inflammatory bowel disease sub-types	Not applicable	CD (15/25), 60%	

CD Crohn's disease; Significant *p* values are bolded; IBD inflammatory bowel disease; LI-L4 first to fourth lumbar vertebrae

Figure Legends

Figure 1:

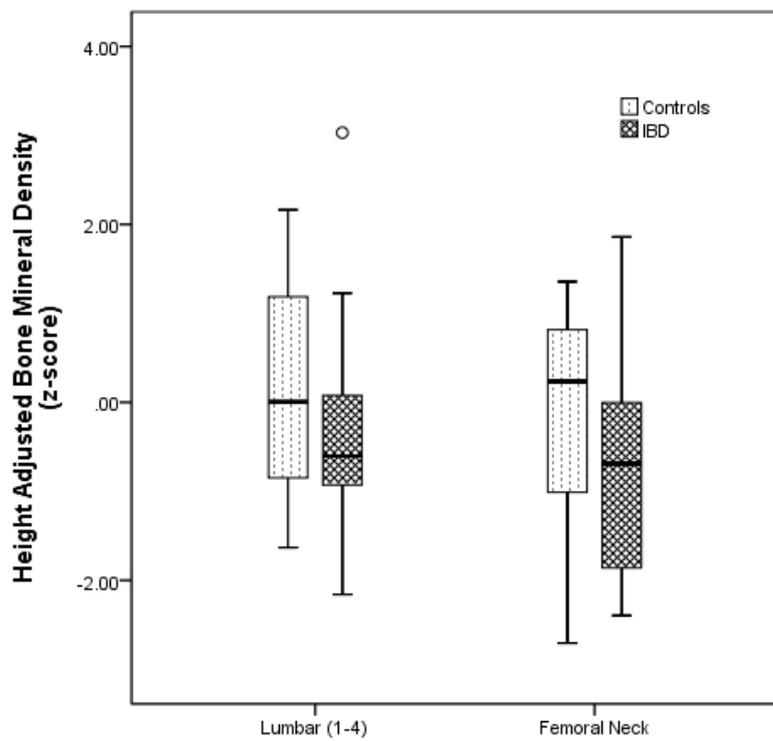
Box plot of the comparison of height-adjusted femoral neck and lumbar bone mineral density z-scores between subjects with inflammatory bowel disease (IBD) and controls. Height-adjusted BMD z-scores were non-significantly lower in the IBD subjects vs. controls for both the femoral neck (-0.8 ± 1.1 vs. -0.06 ± 1.1 , $p=0.070$) and lumbar vertebrae (-0.4 ± 1.2 vs. 0.2 ± 1.2 , $p=0.086$).

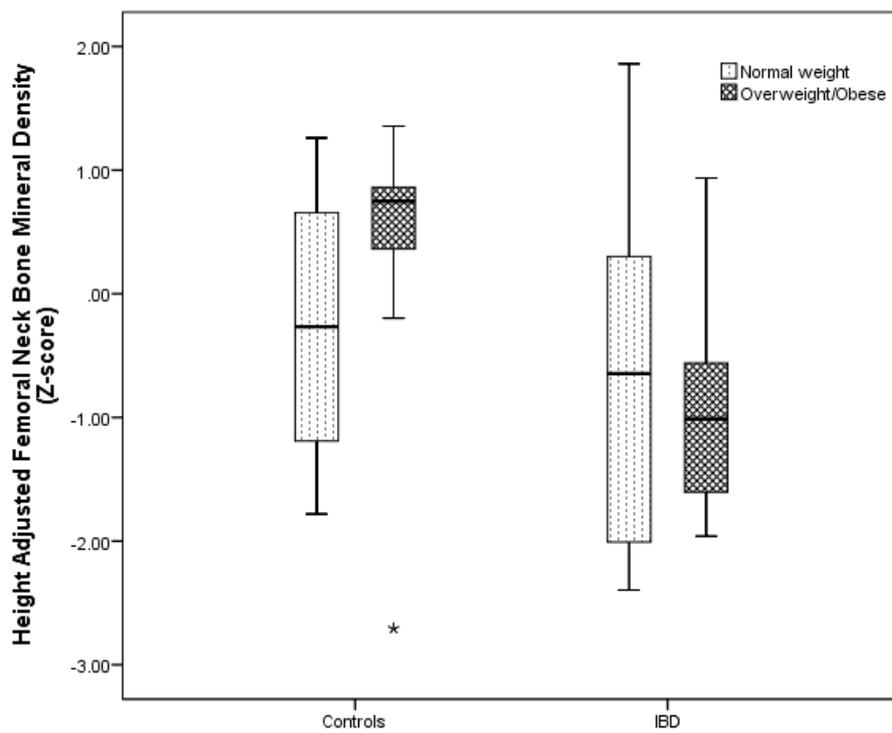
Figure 2:

Box plot of the comparison of height-adjusted femoral neck bone mineral density z scores between subjects with inflammatory bowel disease (IBD) and controls stratified by body mass index (BMI) into normal weight (BMI <85th percentile) or overweight/obesity (BMI ≥85th percentile). BMD z-score was significantly lower in the overweight/obese IBD subjects compared to overweight/obese controls (-0.9 ± 0.9 vs. 0.3 ± 1.3 , $p=0.032$); but was similar between the normal-weight controls and the normal-weight IBD subjects (-0.3 ± 1.0 vs. -0.7 ± 1.3 , $p=0.310$). There was no significant difference in BMD z-score between the normal-weight vs. overweight/obese controls (-0.3 ± 1.0 vs. 0.3 ± 1.3 , $p=0.249$) on one hand, and the normal-weight subjects with IBD vs. overweight/obese subjects with IBD (-0.7 ± 1.3 vs. $0.3 \pm -0.9 \pm 0.9$, $p = 0.600$) on the other. * indicates values >2SD from the mean.

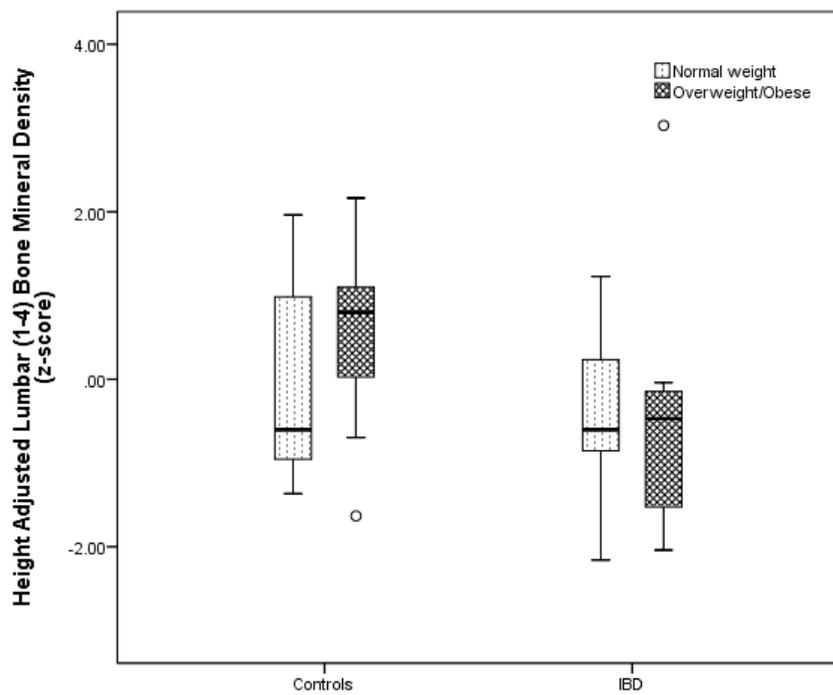
Figure 3:

Box plot of the comparison of height-adjusted lumbar spine bone mineral density (BMD) z-scores between subjects with inflammatory bowel disease (IBD) and controls stratified by body mass index (BMI) into normal weight (BMI <85th percentile) or overweight/obesity (BMI ≥85th percentile). There were no significant differences in height-adjusted BMD z-scores between the normal-weight vs. overweight/obese controls (-0.01 ± 1.2 vs. 0.5 ± 1.3 $p = 0.306$); or normal-weight vs. overweight/obese subjects with IBD (-0.4 ± 1.0 vs. -0.4 ± 1.6 , $p=0.991$). There was equally no difference between the normal-weight controls vs. normal-weight IBD (-0.01 ± 1.2 vs. -0.4 ± 1.0 , $p=0.32$). The difference between the overweight/obese IBD subjects compared to overweight/obese controls (-0.4 ± 1.6 vs. 0.5 ± 1.3 , $p=0.197$) did not reach statistical significance.





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HIGHLIGHTS

1. Adiposity was associated with reduced bone mass in the early phases of inflammatory bowel disease (IBD), but with increased bone mass in the controls.
2. Bone mass did not differ between the normal-weight IBD subjects and normal-weight controls in the early phases of IBD in children and adolescents.
3. Bone mass was lower in children and adolescents with Crohn's disease compared to those with ulcerative colitis.