HHS Public Access

Author manuscript

*J Autism Dev Disord*. Author manuscript; available in PMC 2015 March 24.

Published in final edited form as:

*J Autism Dev Disord*. 2013 July ; 43(7): 1623–1629. doi:10.1007/s10803-012-1709-3.

# Bone Density in Peripubertal Boys with Autism Spectrum Disorders

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## Abstract

We determined whether bone mineral density (BMD) is lower in boys with autism spectrum disorders (ASD) than controls, and also assessed variables that may affect BMD in ASD. BMD was measured using dual energy X-ray absorptiometry (DXA) in 18 boys with ASD and 19 controls 8–14 years old. Boys with ASD had lower BMD Z-scores at the spine, hip and femoral neck, and differences at the hip and femoral neck persisted after controlling for maturity and BMI. Vitamin D intake from food and in serum were lower in ASD subjects, as was exercise activity. We conclude that BMD is lower in peripubertal boys with ASD and may be associated with impaired vitamin D status and lower exercise activity.

### Keywords

Autism; Autism spectrum disorder; Bone density; Puberty; Bone turnover; Bone metabolism



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## Introduction

Autism spectrum disorders (ASD) are a group of behaviorally-defined disorders characterized by impaired social interactions, verbal and non-verbal communication, and repetitive phenomena along with unusual behavior or play (American Psychiatric Association 2000). The prevalence of ASD in American children was recently reported to be increased by the Autism and Developmental Disabilities Monitoring Network (CDC), now affecting 1 in 88 (2009).

The childhood and adolescent years are a critical time for bone accrual towards achievement of peak bone mass, an important determinant of future bone health. Factors that can affect bone accrual during preadolescence and adolescence include genetics, nutritional status [particularly calcium, vitamin D and protein intake (Davies et al. 2005; Foo et al. 2009; Lehtonen-Veromaa et al. 2002)], exercise activity, endocrine alterations and use of specific medications. Low bone density has been reported in various conditions of undernutrition in children, including anorexia nervosa (Misra et al. 2004), celiac disease (Heyman et al. 2009), inflammatory bowel disease (Schmidt et al. 2009) and cystic fibrosis (Grey et al. 2008). Therefore, dietary behaviors and nutritional status of children with ASD are potentially relevant to their bone accrual and risk for low bone mineral density (BMD). Children with ASD often have an unusually restricted diet (including gluten-free casein-free (GFCF) diets, gluten-free (GF) diets and lactose-free diets), which might limit calcium or vitamin D intake.

Children with ASD have high rates of co-morbid neurologic and psychiatric illnesses, including epilepsy and mood disorders, which may be associated with increased cortisol levels, exacerbating the risk for low bone density (Greaves-Lord et al. 2009; Lopez-Duran et al. 2009; Sheth et al. 2008). Children with ASD and seizures may also be treated with anticonvulsant medications, some of which impact vitamin D metabolism and BMD (Chou et al. 2007; Pack et al. 2008). Pubertal bone accrual is dependent upon (a) rising levels of bone anabolic hormones such as growth hormone (GH) and insulin like growth factor-1 (IGF-1) (secreted by the liver and locally at target tissues in response to GH), (b) rising levels of anti-resorptive hormones, such as the sex steroids, and (c) optimal weight bearing activities (Davies et al. 2005). Children with ASD may have impaired GH secretion (Ragusa et al. 1993), with adverse effects on IGF-1 synthesis. The nutritionally dependent process of IGF-1 synthesis (Soyka et al. 1999) is also at risk in children with dietary anomalies. Finally, low muscle tone (Ming et al. 2007) and low exercise rates (Pan 2008) in children with ASD may affect bone development.

There is no comprehensive analysis thus far examining the relationship between autism and BMD. One study (Hediger et al. 2008) reported decreased cortical bone thickness using radiographs in children with autism or ASD, raising concerns regarding the impact of these disorders on bone mass and bone mineral acquisition. The objectives of this study were to determine whether BMD, as assessed with dual energy X-ray absorptiometry (DXA), is lower in boys with ASD than in controls (boys with no other diseases that may affect bone metabolism), and what differences there are between groups for factors that might be causally related to BMD. We hypothesized that BMD would be lower in boys with ASD

than in controls, and would be associated with lower dietary calcium and vitamin D intake, and lower levels of serum IGF-1 and testosterone.

## Methods

### Subjects

Eighteen boys with ASD and 19 controls between the ages of 8–14 years participated in the study. All affected subjects met Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) and Autism Diagnostic Observation Schedule criteria for an ASD (Lord et al. 2000; Lord et al. 1994), and had a body mass index (BMI) between the 3rd–97th percentiles for age. No subject was on medications known to directly affect bone metabolism including testosterone, estrogen/progesterone preparations or glucocorticoids (except local application). No subject was on anticonvulsants that affect BMD such as diphenylhydantoin, phenobarbital, topiramate, carbamazepine and valproic acid. No child had a disease known to affect BMD, including Crohn's disease, celiac disease, thyroid and renal disease. The control group of 19 boys without ASD (ages 8–14 years) was recruited by advertisements in pediatricians' offices, on the Internet and by word of mouth. Our subjects self-identified their race as follows: 32 Caucasian, three African-American, one Asian and one mixed. The Institutional Review Board of Partners Health Care approved the protocol. Informed assent and consent were obtained from subjects and their parents, respectively.

### Procedures

All subjects were evaluated during an outpatient visit at the Clinical Research Center at Massachusetts General Hospital after a screening telephone call or visit to determine eligibility. Bone age was determined by an X-ray of the left hand and wrist (Greulich and Pyle 1959). Height was measured using a single stadiometer (average of triplicate measurements), and weight was measured on an electronic scale. Age and gender norms were determined for height and BMI (Ogden et al. 2002). A salivary cortisol sample was collected from each subject at 8 a.m. and 11 p.m. (when cortisol levels peak and nadir, respectively, in a healthy population). Fasting serum samples were obtained for calcium, phosphorus, 25(OH) vitamin D [25(OH)D], testosterone and IGF-1. We also measured a marker of bone formation, N-terminal propeptide of Type 1 procollagen (PINP), and a marker of bone resorption, N-telopeptide (NTX), also collected in a fasting state.

BMD of the lumbar (L1–L4) spine and hip was measured by dual energy X-ray absorptiometry (DXA) (Hologic 4500, Waltham, MA, USA). DXA is a standardized, reproducible method of determining BMD. Normative age and gender-based data are available for DXA in children. Because DXA measures areal and not volumetric BMD, it overestimates BMD in tall children and underestimates BMD in short children (Carter et al. 1992). To correct for height, we used measures of bone mineral apparent density (BMAD) for the spine (Wren et al. 2005). In addition, we assessed absolute and height adjusted Z-scores using the database and methods of Zemel et al. (2011).

We used radioimmunoassay to measure 25(OH)D (Diasorin, Inc., Stillwater, MN, USA; intra-assay coefficient of variation (CV) of 4.4–8.3 % and lower limit of detection of 1.5 ng/

dl), PINP (Orion Diagnostica Oy, Espoo, Finland; intra-assay CV of 3.5–5.3 % and lower limit of detection of 0.7 ng/ml). A chemiluminescent immunoassay (Beckman Coulter, Fullerton, CA, USA) was used to measure testosterone (intra-assay CV of 1.67–3.93 %, lower limit of detection 10 ng/dl). IGF-1 was assessed using an enzyme immunoassay (ALPCO Diagnostics, Salem, NH; intra-assay CV of 6.6–9.7 %; lower limit of detection 2.3 ng/ml). NTX was measured using an enzyme immunoassay by Labcorp. Calcium and phosphorus were assessed using standard methods.

Subjects maintained a food record for 3 days. Written and verbal guidelines were provided for estimation of food portions. Participants were encouraged to depict typical food consumption. The CRC research dietitian performed nutrient calculations from the three-day food record using the Minnesota Nutrition Data System (NDS) software (version 4.03; nutrient database 31). Typical exercise activity of subjects was determined using the Youth Physical Activity Survey and categorized as ‘Sedentary’, ‘Low Active’, ‘Active’, and ‘Very Active’) (personal communication from Cincinnati Children’s Hospital Medical Center).

JMP (version 9) was used to analyze the data (reported as means ± SEs). A p value of $<0.05$ was considered to be significant. We based the power analysis on the primary endpoint, the mean difference in BMD in children with ASD versus controls. We estimated that group sample sizes of at least 18 boys would achieve >80 % power to detect a difference of C1 SD between groups at a significance level of 0.05. We compared boys with ASD with controls using the Student t test when data were normally distributed, and the Wilcoxon Rank Sum test when data were not normally distributed. The Fisher’s Exact test was used to compare proportions.

We used analysis of covariance (ANCOVA) to control for bone age when comparing differences between groups for biochemical parameters, calcium and vitamin D intake, and bone density parameters. We also used ANCOVA to control for bone age and BMI SDS when comparing differences between groups for bone density parameters (given that BMI SDS is an important determinant of bone density measures in general). When comparing proportions, to control for bone age, or bone age and BMI SDS, we used logistic regression.

## Results

### Clinical and Anthropometric Data

Boys with ASD did not differ from controls for age, bone age, weight, height, BMI or BMI standard deviation score (SDS) (Table 1). Reported physical activity was lower in subjects with ASD; 11.1, 33.3, 44.4 and 11.1 % of boys with ASD reported sedentary, low active, active, and high activity levels, respectively, compared with 5.3, 5.3, 15.8 and 73.7 % of controls ($p = 0.002$) (Table 2).

### Bone Density and Bone Marker Data

Boys with ASD had lower BMD at the spine, hip and femoral neck than controls (Table 3). Similarly, instrument generated BMD Z-scores at these sites were markedly lower in boys with ASD than controls, as was spine BMAD (height-adjusted measure of bone density). A higher proportion of boys with ASD had BMD Z-scores <-2 at the spine and femoral neck.

Differences between the groups for BMD and BMD Z-scores at the hip and femoral neck persisted after controlling for (a) bone age, and for (b) bone age and BMI SDS (Table 3).

We also assessed Z-scores and height adjusted Z-scores using the database and methods of Zemel et al. (2011). Using this database, spine Z-scores were −0.90 ± 0.35 versus 0.14 ± 0.25 ($p = 0.02$) and total hip Z-scores −0.61 ± 0.27 versus 0.44 ± 0.26 ($p = 0.008$) in boys with ASD versus controls. Height adjusted Z-scores also differed significantly between the groups (Spine height adjusted Z-scores: −1.11 ± 0.31 vs. −0.33 ± 0.21, $p = 0.04$; Hip height adjusted Z-scores: −0.80 ± 0.22 vs. 0.10 ± 0.27, $p = 0.01$).

Serum P1NP was lower and NTX higher in boys with ASD; however, these differences did not reach statistical significance (P1NP: 589 ± 66 vs. 671 ± 64 ng/ml, and NTX: 53.7 ± 4.0 vs. 60.4 ± 5.0 ng/ml in ASD and controls).

## Nutritional Data

Four boys with ASD were on a GFCF diet, one on a glutenfree diet, and another on a dairy-free diet. Five of these six subjects were on supplements. Overall, eight boys with ASD and seven controls were on supplements. Hip BMD and BMD Z-scores, and femoral neck BMD and BMD Z-scores (but not other BMD measures) were significantly lower in four boys with ASD on GFCF diets compared with other boys with ASD (hip BMD: 0.59 ± 0.03 vs. 0.78 ± 0.02 g/cm$^2$, $p = 0.007$; hip BMD Z-scores: −2.10 ± 0.17 vs. −0.66 ± 0.25, $p = 0.02$; femoral neck BMD: 0.51 ± 0.03 vs. 0.63 ± 0.02 g/cm$^2$, $p = 0.01$; femoral neck BMD Z-scores: −2.55 ± 0.38 vs. −1.38 ± 0.20, $p = 0.02$).

Vitamin D intake from food, though not from food and supplements, was lower in boys with ASD (Table 2), as was lactose intake. Compared to controls, a smaller proportion of boys with ASD met the estimated requirement for total daily vitamin D intake (400 IU) and there was a trend towards fewer boys with ASD meeting the Recommended Daily Allowance (RDA) (600 IU) (Slomski 2011). The six boys on restricted diets did not differ from other boys with ASD for vitamin D intake from food. However, their vitamin D intake from supplements was higher ($p = 0.009$).

Total calcium intake was lower in boys with ASD (Table 2). The groups did not differ in total caloric, fat, protein and carbohydrate intake. Similarly, total soluble and insoluble dietary fiber intake did not differ between groups; nor did glucose, fructose, galactose, maltose, sucrose, starch, animal and vegetable proteins, cholesterol, and saturated, monounsaturated and polyunsaturated fatty acids intake.

## Biochemical Data

Levels of calcium, phosphorus, IGF-1, testosterone, free androgen index and AM salivary cortisol did not differ between groups. Serum 25(OH)D levels were lower, and PM salivary cortisol higher in boys with ASD than in controls (Table 1). The six boys with ASD on restricted diets did not differ from other boys with ASD for 25(OH)D levels. Also, 25(OH)D levels did not differ between groups after controlling for season of assessment.

## Discussion

The results of this study indicate that bone mineral density (BMD) is lower in boys with ASD compared with controls at the spine, femoral neck and total hip. These data raise significant concerns regarding bone health in this population, and prospective studies are necessary to determine whether peak bone mass acquisition is deleteriously affected in children with ASD.

In this study, as hypothesized, we found that boys with ASD had lower BMD compared with controls. Total caloric intake and intake of specific macronutrients did not differ between groups. However, intake of vitamin D and lactose from food was lower in boys with ASD and may reflect lower intake of milk and dairy products. Studies have reported a high prevalence of malabsorptive symptoms (Valicenti-McDermott et al. 2008) and decreased lactase activity in children and adults with autism (Kushak et al. 2011; Horvath et al. 1999), and an intolerance to lactose may contribute to this decreased intake. Dietary sources of vitamin D are limited, and much of one's dietary intake of vitamin D is derived from fortified milk and milk products (Misra et al. 2008). Thus, limited dairy intake could significantly impact serum 25(OH)D levels, particularly in the absence of other sources of vitamin D supplementation. Finally, endogenous vitamin D synthesis requires sun exposure, and this may also be limited in children with ASD given their reduced activity levels and time spent outdoors. In our study, serum vitamin D levels were lower in ASD, and a larger proportion of boys with ASD than controls had vitamin D levels <32 ng/ml, the recommended lower limit for optimal vitamin D levels (Holick et al. 2011). Adequate vitamin D intake is essential for optimal bone mineralization and pubertal bone accural (Lehtonen-Veromaa et al. 2002), and a lower vitamin D intake in boys with ASD associated with lower serum 25(OH)D levels in the long-term may have important deleterious effects on bone health. Studies are necessary to determine whether vitamin D supplementation in boys with ASD is effective in improving bone health in this population.

Exercise can have an important impact on bone health (Davies et al. 2005), and boys with ASD had lower activity levels than controls. More studies (using detailed and validated questionnaires) are necessary to examine the impact of exercise activity on bone density in boys with ASD. If future studies indicate that a lower exercise level is an important determinant of bone density in boys with ASD, structured exercise programs may be necessary to optimize pubertal bone accrual in this condition.

Insulin-like growth factor-1 (IGF-1) is a nutritionally regulated hormone important for bone formation during the pubertal years (Russell et al. 2011), and testosterone is another important determinant of pubertal bone metabolism that increases with increasing maturity. Aromatization of testosterone to estrogen decreases bone resorption, while testosterone has direct bone anabolic effects (Leder et al. 2003; Michael et al. 2005). However, consistent with a lack of difference in caloric intake and BMI between boys with ASD and controls, the groups did not differ with regard to serum IGF-1. Similarly, testosterone levels did not differ across groups.

Of importance, total hip and femoral neck bone density Z-scores remained lower in boys with ASD compared with controls, even after controlling for maturity and BMI. The femoral neck is the site most susceptible to fractures when hip bone density is low, and there are likely factors other than those assessed in this study that contribute to bone density at this site. We did observe significantly lower total hip and femoral neck BMD measures in boys with ASD on GFCF diets compared with other boys with ASD, and it is possible that lack of specific nutrients in such diets is a major contributor to low bone density. This will be important to assess in future studies.

Limitations of this study include its cross-sectional nature and the fact that ass ociations do not prove causation; thus causality cannot be inferred from our data. Additionally, we did not collect data regarding use of atypical antipsychotics, which may impact bone density by increasing prolactin secretion and causing hypogonadism (Bostwick et al. 2009). We also excluded children on anticonvulsants that may impact vitamin D metabolism thus limiting generalizability. Prospective studies are necessary to confirm the role of vitamin D status in modifying bone metabolism in children with ASD, and the impact of medications and exercise.

These are the first data to demonstrate that boys with ASD have lower bone density than controls. This is a significant finding and raises major concerns regarding not only the immediate risk for fractures in this population, but also peak bone mass acquisition and long-term bone health. Further prospective studies are necessary to examine bone accrual rates in children with ASD, and the impact of vitamin D intake and exercise on bone density in boys with ASDs.

## Acknowledgments

This research was supported in part by NIH grants 1 UL1 RR025758-01 and as part of the Autism Speaks Autism Treatment Network. Further support came from a cooperative agreement (UA3 MC 11054) from the U.S. Department of Health and Human Services, Health Resources and Services Administration, Maternal and Child Health Research Program, to the Massachusetts General Hospital, and in partnership with the Lurie Center for Autism. The views expressed in this publication do not necessarily reflect the views of Autism Speaks, Inc. We are grateful to our colleagues Drs. Susan McGrew and Cynthia Molloy for their collaboration and help in setting up this project, and Drs. Susan McGrew and James Perrin for their help in preparation of this manuscript. We would also like to thank Dr. Christopher McDougle for his helpful comments in the revision of this manuscript.

## Abbreviations

| | |
|---|---|
| **BMD** | Bone mineral density |
| **BMAD** | Bone mineral apparent density |
| **DXA** | Dual energy X-ray absorptiometry |
| **ASD** | Autism spectrum disorder |
| **IGF-1** | Insulin-like growth factor-1 |
| **TSH** | Thyroid stimulating hormone |
| **T4** | Thyroxine |

| | |
|---|---|
| **P1NP** | N-terminal propeptide of Type 1 procollagen |
| **NTX** | N-telopeptide |
| **SGBG** | Sex hormone binding globulin |
| **SD** | Standard deviation |
| **SE** | Standard error |
| **CV** | Coefficient of variation |

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## Table 1

Clinical characteristics and biochemical parameters in boys with ASD and controls

| | **ASD n = 18** | **Controls n = 19** | ***p* value** | ***p* value*** |
|---|---|---|---|---|
| Age (years) | 10.6 ± 0.4 | 11.2 ± 0.3 | 0.23 | |
| Bone age (years) | 10.6 ± 0.6 | 11.8 ± 0.4 | 0.08 | |
| BMI (kg/m$^2$) | 18.9 ± 1.0 | 17.6 ± 0.40 | 0.24 | 0.11 |
| BMI SDS | 0.26 ± 0.30 | 0.02 ± 0.20 | 0.48 | 0.60 |
| Height SDS | 0.40 ± 0.20 | 1.01 ± 0.25 | 0.06 | 0.17 |
| Biochemical parameters | | | | |
| Calcium (mg/dl) | 9.7 ± 0.1 | 9.6 ± 0.1 | 0.67 | 0.49 |
| Phosphorus (mg/dl) | 4.6 ± 9.1 | 4.7 ± 0.1 | 0.37 | 0.59 |
| 25 (OH) vitamin D (ng/ml) | 26.7 ± 1.9 | 31.7 ± 1.6 | **0.05**** | 0.06 |
| 25(OH) vitamin D <32ng/ml | 76.5 % | 36.8 % | **0.02** | **0.03** |
| IGF-1 (ng/ml) | 165 ± 18 | 173 ± 13 | 0.70 | 0.58 |
| Testosterone (ng/dl) | 90 ± 43 | 119 ± 33 | 0.58 | 0.62 |

Bold values indicate significant differences between groups *SDS* Standard deviation score

* Adjusted for bone age

** Non-parametric testing

## Table 2

Dietary intake, season of examination and exercise classification in boys with ASD and controls

| | ASD n = 18 | Controls n = 19 | *p* value | *p* value* |
|---|---|---|---|---|
| Dietary intake of calcium and vitamin D | | | | |
| Calcium intake from food (mg/d) | 877 ± 77 | 1,149 ± 125 | 0.08 | 0.13 |
| Calcium intake from food and supplements (mg/d) | 878 ± 92 | 1,184 ± 121 | **0.05** | 0.11 |
| % Meeting EAR (calcium intake) | 33.3 % | 57.9 % | 0.19 | 0.25 |
| % Meeting RDA (calcium intake) | 16.7 % | 36.8 % | 0.27 | 0.23 |
| Vitamin D intake from food (IU/d) | 199 ± 26 | 340 ± 56 | **0.03** | **0.04** |
| Vitamin D intake from food and supplements (IU/d) | 314 ± 62 | 489 ± 82 | 0.07** | 0.14 |
| % Meeting EAR (vitamin D intake) | 27.8 % | 68.2 % | **0.049** | **0.05** |
| % Meeting RDA (vitamin D intake) | 5.6 % | 31.6 % | 0.09 | **0.046** |
| Season of examination | | | | |
| Fall/winter | 44 % | 16 % | 0.08 | 0.11 |
| Spring/summer | 56 % | 84 % | | |
| Exercise classification | | | | |
| Sedentary | 11.1 % | 5.3 % | 0.002 | <0.0001 |
| Low activity | 33.3 % | 5.3 % | | |
| Active | 44.4 % | 15.8 % | | |
| High activity | 11.1 % | 73.7 % | | |

Bold values indicate significant differences between groups

*SDS* standard deviation score, *EAR* estimated average requirement, *RDA* recommended dietary allowance

* Adjusted for bone age

** Non-parametric testing

**Table 3**

BMD in children with ASD and controls

| | **ASD n = 18** | **Controls n = 19** | ***p*** | ***p*** * | ***p*** ** |
|---|---|---|---|---|---|
| Lumbar spine BMD (g/cm$^2$) | 0.56 ± 0.03 | 0.66 ± 0.02 | **0.02** | 0.12 | **0.047** |
| Lumbar spine BMD Z-score | −1.13 ± 0.28 | −0.21 ± 0.25 | **0.02** | 0.06 | **0.02** |
| Lumbar spine BMD Z-scores <−2 | 27.8 % | 0 % | **0.02** | **0.02** | **0.03** |
| Lumbar spine BMAD (g/cm$^3$) | 0.09 ± 0.00 | 0.11 ± 0.00 | **0.02** | 0.13 | 0.06 |
| Femoral neck BMD (g/cm$^2$) | 0.60 ± 0.02 | 0.72 ± 0.02 | **0.0005** | **0.003** | **0.001** |
| Femoral neck BMD Z-score | −1.64 ± 0.21 | −0.52 ± 0.24 | **0.001** | **0.003** | **0.001** |
| Femoral neck BMD Z-scores <−2 | 33.3 % | 0 % | **0.008** | **0.005** | **0.006** |
| Hip BMD (g/cm$^2$) | 0.70 ± 0.02 | 0.81 ± 0.02 | **0.002** | **0.008** | **0.003** |
| Hip BMD Z-score | −0.92 ± 0.24 | 0.14 ± 0.29 | **0.009** | **0.007** | **0.002** |
| Hip BMD Z-scores <−2 | 16.7 % | 5.3 % | 0.34 | 0.26 | 0.24 |

Bold values indicate significant differences between groups

* *p* adjusted for bone age

** *p* adjusted for bone age and BMI SDS