Bone Accrual in Males with Autism Spectrum Disorder

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Objective To test the hypothesis that bone accrual over a 4-year period is reduced in boys with autism spectrum disorder (ASD) compared with typically developing controls.

Study design Twenty-five boys with ASD and 24 controls were assessed for bone outcomes. Fourteen boys with ASD and 11 controls were assessed both at baseline and after 4 years. The mean subject age was 11.0 ± 1.6 years at study initiation and 14.9 ± 1.6 years at follow-up. Bone mineral density (BMD) was measured at the spine, hip, and whole body using dual-energy X-ray absorptiometry and normalized for age, race, and sex (BMD *z*-scores). Height adjustments were performed as well. We assessed medical history, physical activity using questionnaires, vitamin D and calcium intake using food records, and serum calcium, phosphorus, 25(OH)-vitamin D, and pubertal hormone levels.

Results Boys with ASD had lower spine, hip, and whole body BMD *z*-scores compared with controls. In those subjects assessed both at baseline and after 4 years, bone accrual rates did not differ between the 2 groups; however, spine and hip BMD *z*-scores remained lower in the boys with ASD than in controls at follow-up. Notably, the ASD group was less physically active at both time points.

Conclusion Although pubertal bone accrual was similar to that in controls, BMD in children with ASD remained low over a 4-year follow-up period, suggesting that low BMD is a consequence of prepubertal factors, such as low physical activity. Studies are needed to investigate the causes and consequences of decreased BMD, to assess BMD in females and adults with ASD, and to evaluate therapeutic interventions. *(J Pediatr 2017;181:195-201).*

Bone accrual occurs predominantly in childhood and adolescence, with 40% of peak bone mass accumulated during puberty.[1,2] In a cross-sectional study, our group reported decreased bone mineral density (BMD) at the spine, hip, and femoral neck in peripubertal boys with autism spectrum disorder (ASD) compared with typically developing peripubertal age-matched male controls.[3] Other groups also have reported low BMD *z*-scores[4] and decreased bone cortical thickness in ASD.[5] Nonetheless, little is known about prospective bone accrual rates during puberty in children with ASD compared with typically developing controls. Greater bone accrual during puberty in children with ASD could allow these children to catch up to controls. Given the limited potential for bone accrual in adulthood, failure to catch up during adolescence will lead to low peak bone mass and potentially increased lifetime fracture risk. This is particularly relevant in individuals with ASD, given the elevated risk of hip and other fractures in children and adults with ASD compared with controls (OR of 3.33 in children and 11.7 in adults) found in an evaluation of a national emergency department database.[6]

Certain comorbid conditions seen with ASD may lead to low BMD. These include feeding issues, such as self-imposed restricted diet or a gluten-free or casein-free diet[7,8]; insufficient vitamin D intake[3,9,10]; gastrointestinal symptoms affecting food intake and absorption[11]; co-occurring gastrointestinal diseases, such as colitis[12]; chronic use of medication such as antiepileptics,[13,14] antipsychotics,[15,16] proton pump inhibitors,[17] and selective serotonin reuptake inhibitors[16]; hypotonia[18]; prematurity[19]; and low physical activity levels.[20]

Longitudinal BMD data are lacking in children with ASD. In this study, we sought to estimate bone accrual rates over a 4-year period in prepubertal and peripubertal boys with ASD and typically developing controls, and to determine whether pubertal bone accrual was affected by factors common in children with ASD that may affect BMD, including physical activity and dietary calcium and

25(OH)D	25-hydroxy vitamin D
ASD	Autism spectrum disorder
BMD	Bone mineral density
BMD-CS	Bone Mineral Density in Childhood Study
BMI	Body mass index
CV	Coefficient of variation
IGF-1	Insulin-like growth factor 1
NDSR	Nutrition Data System for Research
NTX	N-telopeptide

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vitamin D intake. We hypothesized that low BMD *z*-scores would persist over the 4-year period, and that the bone accrual rate would be lower in the children with ASD compared with controls. As a secondary aim of this study, we examined whole body BMD, an important predictor of fracture risk in children[21] not previously assessed in children with ASD.

Methods

All children with ASD met the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition*[22] and Autism Diagnostic Observation Schedule criteria for an ASD diagnosis[23,24] at study enrollment. A total of 38 males (19 with ASD and 19 controls) aged 8-17 years were enrolled at initiation of the cross-sectional study in 2011.[3] Eleven of these participants (5 with ASD and 6 controls) were subsequently lost to follow-up. Our recruitment strategy is described in detail in the report of the initial study.[3] An additional 13 new participants (6 with ASD and 7 controls) were recruited at the follow-up visit in 2015 for assessment of additional bone measures (eg, whole body, whole body less the head). The participants with ASD were clinical patients of the Lurie Center for Autism, and controls were recruited through advertisements in primary care providers' offices, the Internet, and the hospital, and by word of mouth.

On analysis, 2 siblings of the original controls who completed a follow-up visit were excluded owing to an abnormally low level (<15 ng/mL) of serum 25-hydroxy vitamin D [25(OH)D], suggesting that they were no longer controls and had a possible condition with the potential to affect bone metabolism. Therefore, prospective bone accrual data were available for 25 original participants (14 with ASD and 11 controls), and a total of 49 participants (25 with ASD and 24 controls, including 2-3 siblings from 5 families) were available for cross-sectional analyses. The 25 returnees from the original cohort did not differ from the 11 nonreturnees with respect to demographics, anthropomorphic measures, biochemical results, hormone levels, diet, or levels of physical activity (**Table I**; available at www.jpeds.com).

All participants had a body mass index (BMI) between the 3rd and 97th percentiles for age and sex based on standard charts.[25] Exclusion criteria at enrollment included receipt of medication known to affect bone metabolism, including testosterone, estrogen/progesterone, glucocorticoids (except inhaled glucocorticoids), and antipsychotic and anticonvulsant medications, such as diphenylhydantoin, phenobarbital, topiramate, carbamazepine, and valproic acid. Children with a known disease affecting bone metabolism, such as Crohn's disease, celiac disease, thyroid disorders, renal disorders, cerebral palsy, and muscular dystrophy, were excluded. At follow-up, 1 participant in the ASD group was taking valproic acid and another was taking aripiprazole. The Institutional Review Board of Partners HealthCare System approved this study. Informed assent and consent were obtained from participants and their parents.

Experimental Protocol

Participants were screened during a clinic visit or by phone and evaluated at the Clinical Research Center at Massachusetts General Hospital. Bone age was assessed using an X-ray of the left hand and wrist.[26] Tanner stage was captured by self-report using standard pictures. Height was calculated as the average of 3 measurements obtained on a single stadiometer, and weight was measured on an electronic scale. Height and BMI *z*-scores were determined using age- and sex-based norms.[25] Percentage body fat and fat-free mass were determined using bioimpedance and Body Composition software version 4.0.0 (RJL Systems, Clinton Township, Michigan). Dual-energy X-ray absorptiometry (Discovery A, with Apex 4.0.2 software; Hologic, Bedford, Massachusetts) was used to measure BMD of the lumbar (L1-L4) spine, total hip, femoral neck, and whole body. The various *z*-scores were assessed using the Hologic database and the database from the longitudinal Bone Mineral Density in Childhood Study (BMD-CS).[27-29] These *z*-scores standardize BMD measurements for age, race, and sex. Height-adjusted BMD *z*-scores were also calculated using the latter database. Bone accrual was calculated for the spine, total hip, and femoral neck as the difference in BMD from the initial visit to the follow-up visit. We also report (for the first time) BMD data for the whole body and the whole body less the head in children with ASD from assessments at the follow-up visit; these measurements were not assessed at the initial visit.

Each participant's parents maintained a food record for 3 days (2 weekdays and 1 weekend day) based on the child's food intake close to time of the clinic visit. Parents were given written instructions and visual estimations of food portions. The records were reviewed by a registered dietitian at the Clinical Research Center, who performed nutrient calculations using the Minnesota Nutrition Data System for Research (NDSR) versions 2009 and 2014, developed by the University of Minnesota's Nutrition Coordinating Center. Final calculations were completed using NDSR version 2014 for all visits. The NDSR time-related database updates analytic data while maintaining nutrient profiles true to the version used for data collection. For this study, we assessed the consumption of food and supplements linked to bone health, such as calcium and vitamin D. The intake of nutrients was further characterized as intake of the nutrient from food, supplements, and both.

Physical activity was determined using the Youth Physical Activity Survey, an unvalidated instrument from Cincinnati Children's Hospital Medical Center that classifies ASD behaviors, such as flapping and categorizes subjects, as "sedentary," "low active," "active," or "very active," and the Oxford Physical Activity Questionnaire to derive metabolic equivalents.[30]

Serum samples were collected for calcium and phosphorus measurements, which were performed by LabCorp (Burlington, North Carolina) following standard clinical methods. Insulin-like growth factor 1 (IGF-1), 25(OH)D, estradiol, and testosterone levels were measured by liquid chromatography–mass spectrometry. The bone formation marker N-terminal propeptide of type 1 procollagen was assayed using a competitive radioimmunoassay kit (Orion Diagnostica UniQ, Espoo, Finland) that has an intra-assay coefficient of variation (CV)