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Bone Microarchitecture in Adolescent Boys with Autism Spectrum Disorder

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Abstract

Background: Boys with autism spectrum disorder (ASD) have lower areal bone mineral density (aBMD) than typically developing controls (TDC). Studies of volumetric BMD (vBMD) and bone microarchitecture provide information about fracture risk beyond that provided by aBMD but are currently lacking in ASD.

Objectives: To assess ultradistal radius and distal tibia vBMD, bone microarchitecture and strength estimates in adolescent boys with ASD compared to TDC.

Design/Methods: Cross-sectional study of 34 boys (16 ASD, 18 TDC) that assessed (i) aBMD at the whole body (WB), WB less head (WBLH), hip and spine using dual X-ray absorptiometry (DXA), (ii) vBMD and bone microarchitecture at the ultradistal radius and distal tibia using high-resolution peripheral quantitative CT (HRpQCT), and (iii) bone strength estimates (stiffness and failure load) using micro-finite element analysis (FEA). We controlled for age in all groupwise comparisons of HRpQCT and FEA measures. Activity questionnaires, food records, physical exam, and fasting levels of 25(OH) vitamin D and bone markers (C-terminal collagen crosslinks and N-terminal telopeptide (CTX and NTX) for bone resorption, N-terminal propeptide of Type 1 procollagen (PINP) for bone formation) were obtained.

Results: ASD participants were slightly younger than TDC participants (13.6 vs. 14.2 years, $p=0.44$). Tanner stage, height z-scores and fasting serum bone marker levels did not differ between groups. ASD participants had higher BMI Z-scores, percent body fat, IGF-1 Z-scores, lower lean mass and aBMD Z-scores than TDC at the WB, WBLH, and femoral neck ($P<0.1$). At the radius, ASD participants had lower trabecular thickness (0.063 vs. 0.070 mm, $p=0.004$), compressive

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Author Contributions

AMN and MM conceptualized the hypothesis and designed the experiments. AMN, NCS, MM, EM, EAM and CJM analyzed the data and wrote the article. NCS, AMN and MM conducted the experiment.

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stiffness (56.7 vs. 69.7 kN/mm, $p=0.030$) and failure load (3.0 vs. 3.7 kN, $p=0.031$) than TDC. ASD participants also had 61% smaller cortical area (6.6 vs. 16.4 mm², $p=0.051$) and thickness (0.08 vs. 0.22 mm, $p=0.054$) compared to TDC. At the tibia, ASD participants had lower compressive stiffness (183 vs. 210 kN/mm, $p=0.048$) and failure load (9.4 vs. 10.8 kN, $p=0.043$) and 23% smaller cortical area (60.3 vs. 81.5 mm², $p=0.078$) compared to TDC. A lower proportion of ASD participants were categorized as “very physically active” (20% vs. 72%, $p=0.005$). Differences in physical activity, calcium intake and IGF-1 responsiveness may contribute to group differences in stiffness and failure load.

Conclusion: Bone microarchitectural parameters are impaired in ASD, with reductions in bone strength estimates (stiffness and failure load) at the ultradistal radius and distal tibia. This may result from lower physical activity and calcium intake, and decreased IGF-1 responsiveness.

Keywords

Bone Mineral Density; Bone Microarchitecture; Autism Spectrum Disorder

1. Introduction

Cross sectional and longitudinal studies have reported that boys with autism spectrum disorder (ASD) have decreased bone mineral density (BMD) at the femoral neck, hip and spine compared to typically developing controls (TDC) [1–3]. Other groups have described decreased bone cortical thickness in boys with ASD using radiography [4]. Children and adults with ASD also have higher odds of hip and other fracture based on a national emergency department database [5]. In this study, among children with ASD, 0.052% of ED visits were for hip fractures and 1.76% for upper limb fractures.

Dual energy x-ray absorptiometry (DXA) is the clinical gold standard for measuring BMD in children and adolescents. Although BMD is an important determinant of fracture risk [6], DXA assesses 2-dimensional, areal BMD (aBMD), underestimating volumetric BMD (vBMD) in short individuals and overestimating vBMD in tall individuals. Also, DXA measures of aBMD do not correlate well with fracture risk [7, 8]. DXA does not assess cortical and trabecular bone microarchitecture [9], parameters known to predict fracture risk independent of aBMD [10, 11]. In contrast, high-resolution peripheral quantitative computed tomography (HRpQCT) measures vBMD and microarchitecture of cortical and trabecular bone [12, 13], and micro-finite element analysis (μFEA) provides estimates of bone strength [10, 14–16]. There are currently no reports of bone microarchitecture and strength estimates in children with ASD.

Since approximately 90% of peak bone mass is gained in the first two decades of life, it is important to optimize bone accrual in childhood and adolescence. In order to optimize pediatric bone accrual, it is important to know the factors that contribute to impaired bone health in any specific condition. Genetics, weight-bearing activity, body habitus, nutritional status, hormonal milieu and medications are important determinants of bone density and structure during childhood and adolescence [17–21]. Children with ASD may be at risk for developing low BMD and impaired bone microarchitecture because of a number of comorbid conditions. These include lower muscle tone, lower exercise levels and unusual or

restricted diets including a gluten- and casein-free diet, or diets low in calcium or vitamin D, such as a dairy-free diet. ASD is also associated with recurring gastrointestinal diseases such as colitis and malabsorption which affect mineral nutrition [22–26]. Finally, children with ASD are often chronically exposed to anti-epileptic [27] and antipsychotic medications [28], selective serotonin reuptake inhibitors (SSRIs) [29], and proton pump inhibitors [30, 31], all of which can have deleterious effects on bone.

The purpose of this study was to examine bone microarchitecture and strength estimates in adolescent boys with ASD compared to TDC and identify factors associated with group differences. We hypothesized that compared with TDC, adolescent boys with ASD would have impaired bone microarchitecture (assessed using HRpQCT) and reduced strength estimates (assessed using μ FEA) and that these differences would be associated with reduced levels of exercise activity and calcium and vitamin D intake.

2. Subjects and Methods

2.1 Subjects:

A total of 40 males ages 9–18 years old (20 ASD and 20 TDC) were enrolled for this cross-sectional analysis. Of the 20 ASD participants, four with missing HRpQCT scan data (because of scheduling conflicts or an inability to stay still long enough for the scan) were excluded from analysis. Additionally, two TDC participants were excluded due to abnormally low levels of serum 25-hydroxy vitamin D (25(OH)D), suggesting possible inflammatory disease with the potential to affect bone metabolism. Therefore, a total of 16 ASD and 18 TDC participants were analyzed. All children had a body mass index (BMI) between the 3rd and 97th percentiles for age based on standard charts [32]. Children with ASD met DSM-IV [33] and Autism Diagnostic Observation Schedule criteria for an ASD [34, 35], and were recruited from the Lurie Center clinical population or through advertisement and word of mouth. The control group was recruited through advertisement in primary care providers' offices, the internet, advertisement within the hospital, and word of mouth.

Exclusion criteria for all participants included use of medications that affect bone metabolism markedly including testosterone, estrogen/progesterone or glucocorticoids (except inhaled glucocorticoids), and use of anticonvulsant medications such as diphenylhydantoin, phenobarbital, topiramate, carbamazepine and valproic acid, which impact vitamin D metabolism. We did not exclude children using other antiepileptic medications from the study given their frequency of use in children with ASD, and because other anti-epileptics do not impact bone metabolism. Also, although we did not exclude patients on antipsychotics, our study sample had no evidence of hyperprolactinemia induced hypogonadism (the primary cause for low bone density associated with such medications). Further, we excluded children with a known disease affecting bone such as Crohn's disease, celiac, thyroid and renal disease as these conditions are known to have marked effects on bone. The Institutional Review Board of Partners HealthCare System approved this study. Informed assent and consent were obtained from subjects and their parents, respectively.

2.2 Experimental Protocol:

All participants had a history and physical examination performed, including self report of pubertal (Tanner) stage using standardized pictures. Calcium and vitamin D intake was assessed by Clinical Research Center dieticians using a 3-day food diary and the Minnesota Nutrition Data System for Research (NDSR) software versions 2009 and 2014, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. Final calculations were completed using NDSR version 2014 for all visits. Food diaries have been validated for use in children [36, 37], and have been used effectively in studies involving children and adolescents [1, 38, 39]. Activity levels were assessed using the Youth Physical Activity Survey (YPAS) and categorized as ‘Sedentary’, ‘Low Active’, ‘Active’, and ‘Very Active’ (personal communication from Cincinnati Children’s Hospital Medical Center). This questionnaire includes some activities more common in ASD such as hopping/rocking and is effective in providing an estimate of the difference in ASD children and TDC for exercise activity. Thus, ‘very physically active’ on this scale may equate to a lower level of physical activity on a different scale. Activities are modeled after the Compendium of Energy Expenditures for Youth [40]. Activities were also assessed using the validated Oxford Physical Activity Questionnaire (OPAQ) [41]. Metabolic equivalents for physical activity were calculated using measures obtained from the Oxford Physical Activity Questionnaire [41].

A fasting morning blood sample was drawn for assessment of serum levels of calcium, phosphorus, 25-hydroxy vitamin D (25(OH)D), testosterone, estradiol, insulin-like growth factor-1 (IGF-1), markers of bone formation (N-terminal propeptide of Type 1 procollagen (P1NP)) and bone resorption (N-telopeptide (NTX) and C-terminal collagen crosslinks (CTX)). 25(OH)D, IGF-1, estradiol and testosterone levels were measured using liquid chromatography–mass spectrometry (at MGH). Serum calcium and phosphorus were assessed using standard assays. P1NP was assessed with a radioimmunoassay kit (Orion Diagnostica UniQ, Espoo, Finland) (intra-assay coefficient of variation (CV) 9.8–10.2%, limit of detection or sensitivity 2 ug/dL), NTX with an enzyme immunoassay (EIA) (Wampole Laboratories, Princeton, NJ) (intra-assay CV 6.9 %, limit of detection or sensitivity 3.2 nM Bone Collagen Equivalents) and CTX with an enzyme-linked immunosorbent assay (Immunodiagnostic Systems, Fountain Hills, AZ) (intra-assay CV 5.2–6.8%, limit of detection or sensitivity 0.02 ng/mL).

Bone mineral density was measured using dual energy x-ray absorptiometry (DXA) (Hologic Discovery A, Software Version: APEX 4.0.2, Bedford, MA USA) at the total hip, femoral neck, lumbar spine (L1–4), whole body and whole body less head (WBLH). Z-scores based on age-, gender-, and race-specific pediatric norms from Hologic are reported. DXA was also used for measures of body composition (lean and fat mass). Bone age was assessed using an x-ray of the left wrist and hand [42].

2.2.1 Bone microarchitecture measurement and micro-finite element

analysis—Volumetric BMD, geometry and microarchitecture were measured in the non-dominant ultradistal radius (non-weight bearing site) (unless there was a history of fracture in which case the dominant side was measured) and distal tibia (weight bearing bone) with

high-resolution peripheral quantitative computed tomography (HRpQCT) using an Xtreme CT from Scanco Medical AG (Basserdorf, Switzerland) with an isotropic voxel size of 82 μm [10]. The precision is 0.7%–1.5% for densities and 2.5%–4.4% for trabecular and cortical microarchitecture. Detailed cortical bone analysis performed by a semi-automated segmentation technique was used for detailed cortical bone analysis.

Linear micro-finite element analysis was performed using the 3D HR-pQCT images (SCANCO Medical XtremeCT; Image Processing Language (IPL) and its FE extension IPLFE); apparent biomechanical properties were calculated under uniaxial compression, as described [14–16, 43–45]. After image segmentation to identify bone from non-bone voxels, each HR-pQCT bone voxel was converted to a hexahedral finite element having linear-elastic and isotropic material behavior, with a Young's modulus and Poisson's ratio of 10 GPa and 0.3, respectively. Boundary conditions simulated frictionless uniaxial compression of the region of interest. Failure load (kN) was estimated by scaling the resultant load from a 1% apparent compressive strain until 2% of all elements reached an effective strain > 7000 μstrain , per published methods [46]. These FEA-derived estimates of failure load are associated strongly with experimentally measured failure loads causing Colles' fractures in cadaveric radii ($r^2 = 0.75$) [46]. HRpQCT and strength estimation by μFEA were done in a single instrument by one operator.

Motion artifact was graded manually by the technician as follows: 1 = excellent, no motion artifact; 2 = good, limited motion artifact; 3 = severe motion artifact. All scans with a score of 3 (five in the ASD group and one in the TDC group for the distal radius, none for the distal tibia) were excluded from the analysis. Of the scans included in the analysis (score of 1 or 2), two radius scans (both in boys with ASD) and two tibia scans (one in a boy with ASD and one in a TDC) had a quality score of 2 (p not significant); all others had a quality score of 1.

2.3 Statistical Analysis:

Data were analyzed using SAS (version 9.4, Cary, NC). Continuous variables were assessed for normality, and natural log transformations were performed to approximate normality as appropriate. We used generalized estimating equations (GEE) to compare ASD vs. TDC participants to account for correlation between siblings as our sample included 5 sets of 2–3 siblings (both control and ASD). To account for effects of season on differences in 25(OH)D at baseline, we adjusted for the sine of Julian day times $-2\pi / 365$ in analyses of 25(OH)D levels. Comparisons of bone microarchitecture between groups are reported after adjusting for age as population-based and age-specific means and standard deviations (SD) were not available to calculate Z-scores. We also performed sensitivity analyses adjusting for known determinants of bone parameters such as BMI Z-scores, activity levels and calcium intake (factors that differed or trended to differ across groups). To formally assess whether activity levels and calcium intake mediated deficiencies in bone parameters in ASD compared to TDC, we ignored potential between-sibling correlation and employed a causal model for direct and indirect effects [47], taking the total natural indirect effect as a measure of mediation. GEE was also used to test associations between bone parameters and covariates. All continuous data are reported as model-estimated means and standard errors and all

categorical data are reported as model-estimated rates and 95% confidence intervals. Findings with p-values <0.10 are reported given the exploratory nature of this analysis and given that data from several participants could not be used because of motion artifact.

3. Results

The two study groups were similar with respect to bone age, height, and biochemical measurements (serum vitamin D, serum calcium, and phosphorous). The proportion of boys at Tanner stages I, II, III, IV and V were 12.5, 12.5, 6.3, 18.8 and 50% in ASD and 16.7, 5.6, 16.7, 27.8, 28.8% in TDC respectively (one not reported) ($p=0.40$). Compared to TDC, ASD participants were approximately 7 months younger on average, although the difference was not significant. Bone age relative to chronological age was approximately 8 months older in ASD compared to TDC on average ($p=0.081$). ASD participants had higher percent body fat ($p=0.032$), lower lean mass ($p=0.092$), and a higher mean BMI Z-score by 0.62 standard deviations ($p=0.083$). One outlier IGF-1 Z-score of -2.4 in the ASD group was omitted from analyses. IGF-1 Z-scores were higher in the ASD group compared to TDC ($p<0.001$), while total testosterone and estradiol showed no differences between groups (Table 1).

Table 2 compares measures of physical activity, dietary intake of calcium and vitamin D, bone mineral density, and bone markers between ASD and TDC. ASD participants tended to be less physically active than their TDC counterparts. Estimated average requirement (EAR) of calcium intake was met by only 40% of ASD participants compared to 72.2% of TDC ($p=0.070$). Vitamin D intake did not differ between the two groups. For all areas assessed for aBMD, mean Z-scores were at least half of a standard deviation lower in the ASD group than the TDC group. ASD participants had significantly lower BMD Z-scores than TDC at the total hip, femoral neck, whole body and whole body less head ($p<0.1$). Bone mineral content at the femoral neck and whole body were lower in ASD than TDC participants and are not reported ($p=0.05$). Bone markers, namely CTX, NTX, and P1NP, did not differ between groups.

Eleven ASD and 17 TDC participants had microarchitecture data at the radius; all 16 ASD and 18 TDC had microarchitecture data at the tibia. Comparisons of bone microarchitecture between study groups after adjusting for age are presented in Table 3. At the radius, cortical area and thickness were 61% lower ($p=0.051$ and 0.054 , respectively) in the ASD group compared to TDC. ASD participants also had lower trabecular thickness ($p=0.004$), compressive stiffness ($p=0.030$), and failure load ($p=0.031$) compared to TDC. At the tibia, cortical area was 23% lower in the ASD group compared to TDC ($p=0.078$). ASD participants were also impaired compared to TDC with respect to compressive stiffness ($p=0.048$) and failure load ($p=0.043$). (We performed a sensitivity analysis after excluding scans with a quality score of 2, and our results overall did not change. At the distal radius, differences weakened for cortical area and thickness, but held for trabecular thickness, compressive stiffness, and failure load. At the distal tibia, differences weakened in magnitude but failure load held significance (details not reported)).

After also adjusting for BMI Z-scores, all aforementioned differences persisted ($p<0.08$) at the radius and became more significant at the tibia ($p<0.05$).

After adjusting for physical activity, represented by the log-transformed metabolic equivalents estimated from the Oxford Physical Activity Questionnaire, the estimates of differences in stiffness and failure load were reduced. In an age-adjusted analysis of mediation by physical activity of the association between ASD status and bone strength estimates, total natural indirect effects were large but not statistically significant: 5.3 kN/mm (95% CI -15.5, 4.8 kN/mm; $p=0.30$) of the overall 13.0 kN/mm compressive stiffness deficit (41%) at the radius and 6.8 kN/mm (95% CI -22.4, 8.7; $p=0.39$) of the overall 27.0 kN/mm deficit (25%) at the tibia. For failure load, physical activity mediated -0.25 kN (95% CI -0.75, 0.24; $p=0.31$) of the overall 0.64 kN deficit (39%) in ASD vs. TDC at the radius and -0.31 kN (95% CI -1.06, 0.43; $p=0.41$) of the overall 1.39 kN deficit (22%) at the tibia.

After adjusting for log-transformed calcium intake, differences between groups for stiffness and failure load weakened at the radius and remained significant at the tibia. Mediation by calcium intake of the association between ASD status and bone strength estimates were modest at the radius (20% to 24%) and negligible at the tibia (-6 to -3%) and none were significant ($p>0.3$).

Relationships between strength estimates and patient characteristics within each study group are presented in Table 4. At the radius, both compressive stiffness and failure load were positively associated with lean mass within both groups. Within the TDC group alone, strength estimates were positively associated with calcium intake, estradiol, total testosterone levels, and IGF-1 Z-scores. No relationships were observed with percent body fat, Vitamin D intake, or physical activity levels in either study group.

At the tibia, both compressive stiffness and failure load were positively associated with lean mass, total testosterone, and estradiol levels. In the TDC group only, compressive stiffness and failure load were also positively associated with percent body fat, calcium intake, IGF-1 Z-scores, and physical activity levels. These relationships were not observed in the ASD group. Again, no relationships were observed between microarchitecture variables and vitamin D in either study group (Table 4).

4. Discussion

This is the first study to evaluate bone microarchitecture and strength estimates in adolescent boys with autism spectrum disorder compared with typically developing controls. The results of the study indicate that trabecular and cortical thickness is decreased at the radius, and strength estimates (compressive stiffness and failure load) are lower at both the radius and tibia in boys with ASD compared with TDC of the same age. Lean mass is positively associated with strength estimates at both sites in both groups. Additionally, at the ultradistal radius in TDC (but not in ASD participants), strength estimates are positively associated with estradiol and testosterone levels and calcium intake. At the distal tibia, strength estimates are positively associated with both estradiol and testosterone levels in both groups. In TDC (but not in ASD participants), strength estimates are positively associated with IGF-1 levels, calcium intake and activity levels. Our data provide an understanding of specific alterations in bone parameters that contribute to the known increase in fracture risk in ASD.

Bone microarchitecture and μ FEA estimates of bone strength are reported to predict fracture risk independent of DXA measures of areal BMD [10, 13–16]. In this study, boys with ASD were found to have lower cortical and trabecular thickness at the ultradistal radius compared to TDC, measures known to be associated with strength estimates in other populations [48–51]. Importantly, strength estimates were lower at both the ultradistal radius and distal tibia in boys with ASD compared with TDC and this difference strengthened at the distal tibia after controlling for age and BMI-Z scores. Nutritional status is an important determinant of bone health [52], and BMI Z-scores reflect nutritional status. Thus higher BMI Z-scores may protect boys with ASD from decreased bone strength.

Furthermore, differences in strength estimates between groups were markedly reduced after controlling for physical activity levels. Although estimates of the mediation by physical activity were not significant, possibly due to the small sample size, they suggest that physical activity may contribute to approximately 40% of the observed deficits at the radius and approximately a quarter of the observed deficits at the tibia. Physical activity and consequent mechanical loading are a key determinant of bone health, particularly in the pre- and peripubertal years [53–55], and our previous [1] and current study indicate lower prevalence of high levels of physical activity in boys with ASD compared with TDC. One potential cause of lower levels of physical activity in children with ASD is hypotonia, which also independently contributes to impaired bone health from decreased pull of muscle on bone [50, 51, 53]. Lower physical activity levels (particularly a decrease in high levels of physical activity) are likely an important cause of impaired bone health in children with ASD, and this is further suggested by the reduction in differences in strength estimates between groups after controlling for physical activity levels.

Overall, lean mass was positively associated with strength estimates in both groups at both sites, and this is consistent with data from studies in other populations [51, 56, 57], and the concept that muscle pull on bone has bone anabolic effects [53]. In addition, physical activity levels were associated positively with strength estimates at the distal tibia, a weight-bearing site, in TDC. It would be expected for a weight-bearing site (such as the tibia) to be more significantly affected by mechanical loading than a non-weight-bearing site (such as the radius). However, this association of physical activity levels with tibial strength estimates was not observed in children with ASD, possibly because of the limited range of physical activity in this group.

Calcium intake, but not vitamin D intake, trended lower in children with ASD than TDC, and calcium intake, but not vitamin D intake, was associated positively with strength estimates in TDC. Whereas calcium is essential for bone mineralization given that bone consists of calcium phosphate deposited on a framework of collagen, vitamin D is important for dietary calcium absorption from the gut. Importantly, after our initial paper of bone density in children with ASD compared with TDC showed significant differences in vitamin D intake across groups [1], greater attention was paid to vitamin D status of ASD patients in our clinic. However, calcium intake was not consistently addressed [58].

As expected for pubertal children, strength estimates correlated positively with levels of gonadal hormones and IGF-1. Levels of the gonadal hormones and IGF-1 increase markedly

during puberty [59] and have important effects on bone [60]. Estradiol primarily inhibits osteoclastic bone resorption (particularly endosteal bone resorption) and affects trabecular more than cortical bone. Testosterone inhibits bone resorption and also stimulates bone formation, particularly periosteal bone apposition. IGF-1 is bone anabolic and stimulates periosteal bone apposition. The lack of a positive association of gonadal hormones and IGF-1 with strength estimates in children with ASD, despite a lack of difference in levels of these hormones compared to TDC, may suggest that other factors (such as low levels of physical activity and low calcium intake) may attenuate the beneficial effects of gonadal hormones and IGF-1 on bone strength estimates. Further, the lack of variability in these measures in the ASD group may have prevented us from observing the associations we saw in the control group.

Our study was limited by its cross-sectional nature, small size and potentially imperfect matching of ASD and TDC participants for factors independent of their ASD status. We had limited power to adjust for multiple potential confounders. Information regarding food and supplement intake and exercise activity was obtained using food diaries and an exercise questionnaire, which may result in inaccurate reporting. Of note, in this study, parents were asked to maintain the food diary and fill out the exercise questionnaire, which we hope resulted in more accurate reporting. Additionally, boys with ASD were more likely to have motion artifacts at the radius, leading to our inability to include data for five of our participants in our analyses for the radius. Thus, this population may face unique challenges with the use of HRpQCT to assess bone size and microarchitecture. We used a training picture story and mock scanners to optimize HRpQCT data acquisition for our study participants. The training picture story included specific information about what to expect during the study visit depicted as pictures. Such picture stories are commonly used to help decrease situational anxiety in children with autism. However, additional measures may be necessary to reduce motion artifact. Of note, at this time HRpQCT remains a research tool and is only available for appendicular sites (QCT at axial sites is associated with a significant amount of radiation), and DXA measures of BMD remain the standard of care for clinical bone assessments in children. Finally, the study included only adolescent boys, and future studies are necessary that include girls as well as a wider age range of participants.

5. Conclusion

We observed that boys with ASD had weaker bones than matched TDC participants. While DXA scans indicate lower aBMD measures in children with ASD at sites such as the hip and whole body, HRpQCT allows determination of differences in bone geometry and microarchitecture that may contribute to reduced bone strength at the appendicular skeleton, such as reduced cortical and trabecular thickness at the radius, and reduced cortical cross-sectional area at the tibia. Studies are necessary to determine whether HRpQCT parameters and assessment of strength estimates using FEA can determine which children with ASD are at an increased risk for fracture. At this time, such assessments are used primarily in a research setting. However, as more data become available in children, it is possible that these assessments will be used clinically to determine fracture risk. Further, lower strength estimates among ASD participants appear to be related to lower levels of physical activity, lower calcium intake and reduced responsiveness to hormones such as IGF-1 in children

with ASD. Interventional studies are necessary to determine whether increasing physical activity levels and calcium intake in children with ASD improves measures of bone health.

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Abbreviations

BMD	bone mineral density
ASD	autism spectrum disorder
HRpQCT	high resolution peripheral quantitative computer tomography
DEXA	dual-energy x-ray absorptiometry
IGF-1	insulin-like growth factor-1
TSH	thyroid stimulating hormone
T4	thyroxine
P1NP	N-terminal propeptide of Type 1 procollagen
NTX	N-telopeptide
GI	Gastrointestinal
SD	standard deviation
SE	standard error
CV	coefficient of variation

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Clinical characteristics of participants with autism spectrum disorder (ASD) and typically developing controls (TDC)

Table 1.

Variable	ASD Mean (SE) or Rate (95% CI)	TDC Mean (SE) or Rate (95% CI)	ASD vs. TDC Difference (95% CI) or OR (95% CI)	P-Value
Chronological Age (years)	13.6 (0.53)	14.2 (0.56)	-0.6 (-2.1, 0.9)	0.44
Bone Age (years)	14.8 (0.49)	14.8 (0.51)	0.1 (-1.3, 1.5)	0.90
Bone Age-Chronological Age (years)	1.2 (0.30)	0.5 (0.25)	0.7 (-0.1, 1.5)	0.081
Tanner stage III or greater	75.0% (48.6, 90.5)	76.5% (51.7, 90.8)	OR=0.9 (0.2, 4.5)	0.92
Height Z-Score	0.58 (0.29)	0.65 (0.32)	-0.07 (-0.90, 0.76)	0.86
BMI Z-Score	0.48 (0.28)	-0.14 (0.23)	0.62 (-0.08, 1.32)	0.083
Percent body fat (%)	29.2 (3.44)	20.9 (1.90)	8.4 (0.7, 16.0)	0.032
Fat mass (kg)	18.8 (3.15)	13.4 (2.07)	5.5 (-1.9, 12.8)	0.14
Lean mass (kg)	8.0 (0.40)	8.9 (0.34)	-0.9 (-1.9, 0.1)	0.092
Serum Vitamin D (ng/mL) (Adjusting for seasonality)	24.9 (1.13)	30.6 (3.41)	-5.7 (-12.8, 1.4)	0.12
Vitamin D insufficiency (levels < 32 ng/mL) (Adjusting for seasonality)	90.9% (63.2, 98.3%)	81.8% (43.5, 96.3%)	OR=2.2 (0.3, 19.1)	0.46
Vitamin D deficiency (levels < 20 ng/mL) (Adjusting for seasonality)	13.2% (3.2, 41.0%)	11.1% (2.6, 36.5%)	OR=1.2 (0.1, 10.4)	0.85
Serum calcium (mg/dL)	9.7 (0.08)	9.7 (0.05)	0.0 (-0.2, 0.2)	0.95
Serum phosphorous (mg/dL)	4.4 (0.17)	4.4 (0.15)	0.0 (-0.4, 0.5)	0.92
Serum estradiol (pg/mL)	17.9 (2.29)	17.7 (1.72)	0.2 (-5.4, 5.9)	0.94
Serum total testosterone (ng/dL)	290.2 (45.0)	361.6 (54.2)	-71.4 (-210.5, 67.6)	0.31
IGF-1 Z-score	0.18 (0.13)	-0.73 (0.16)	0.92 (0.52, 1.31)	<0.001

Table 2.

Physical activity, dietary intake, areal bone mineral density and bone markers in participants with autism spectrum disorder (ASD) and typically developing controls (TDC)

Variable	ASD Mean (SE) or Rate (95% CI)	n	TDC Mean (SE) or rate (95% CI)	n	ASD vs. TDC Difference (95% CI) or OR (95% CI)	P-Value
Youth Physical Activity Level: % Very Active	20.0% (6.6, 47.0)	16	72.2% (48.2, 87.9)	18	OR=0.1 (0.0, 0.5)	0.005
Metabolic Equivalents from the Oxford Physical Activity Questionnaire *	2694.0 (793.0)	16	4256.4 (518.7)	18		
Log-Transformed Metabolic Equivalents from the Oxford Physical Activity Questionnaire	7.4 (0.23)	16	8.1 (0.17)	18	-50.2% (-69.2, -19.4)	0.005
Calcium intake from food (mg/day) *	1103.8 (170.3)	15	1496.1 (161.5)	18		
Log-Transformed Calcium intake from food (mg/day)	6.9 (0.15)	15	7.2 (0.10)	18	-28.7% (-50.5, 2.7)	0.069
Calcium intake from food and supplements (mg/day) *	1130.5 (172.7)	15	1529.5 (147.2)	18		
Log-Transformed Calcium intake from food and supplements (mg/day)	6.9 (0.16)	15	7.2 (0.09)	18	-28.5% (-49.8, 1.9)	0.064
% Meeting EAR (Calcium intake)	40.0% (17.7, 67.4)	15	72.2% (51.2, 86.6)	18	OR=0.3 (0.1, 1.1)	0.070
Vitamin D intake from food (mcg/day) *	7.0 (1.2)	15	9.0 (1.0)	18		
Log-Transformed Vitamin D intake from food (mcg/day)	1.8 (0.19)	15	1.9 (0.14)	18	-16.1% (-47.1, 32.9)	0.45
Vitamin D intake from food and supplements (mcg/day) *	11.7 (2.4)	15	9.8 (1.1)	18		
Log-Transformed Vitamin D intake from food and supplements (mcg/day)	2.2 (0.22)	15	2.0 (0.14)	18	19.2% (-28.0, 97.4)	0.50
% Meeting EAR (Vitamin D intake)	46.7% (22.8, 72.2)	15	38.9% (23.6, 56.8)	18	OR=1.4 (0.4, 4.9)	0.62
Total Hip BMD Z-Score	-0.73 (0.23)	16	-0.23 (0.20)	18	-0.50 (-1.09, 0.09)	0.094
Femoral Neck BMD Z-Score	-1.19 (0.22)	16	-0.41 (0.21)	18	-0.78 (-1.37, -0.19)	0.010
Lumbar Spine BMD Z-Score	-0.62 (0.27)	16	-0.11 (0.19)	18	-0.51 (-1.15, 0.14)	0.12
Whole Body BMD Z-Score	-1.25 (0.20)	16	-0.49 (0.18)	18	-0.75 (-1.27, -0.23)	0.005
Whole Body Less Head BMD Z-Score	-1.12 (0.22)	16	-0.48 (0.19)	18	-0.64 (-1.20, -0.07)	0.029
C-terminal collagen crosslinks (CTX)	2.1 (0.20)	15	2.0 (0.18)	18	0.0 (-0.5, 0.6)	0.88
N-telopeptide (NTX)	54.8 (8.56)	15	48.8 (4.61)	18	5.9 (-13.3, 25.1)	0.55
PINP	576.6 (80.7)	15	513.7 (63.3)	18	62.9 (-140.5, 266.2)	0.54

EAR: estimated average requirement; BMD: bone mineral density; PINP: N-terminal propeptide of Type 1 procollagen

* Comparisons between groups performed using natural log transformed data (log transformations were performed to better approximate normality)

Bone microarchitecture at the ultradistal radius and distal tibia in autism spectrum disorder (ASD) and typically developing controls

Table 3.

Variable	Primary Analysis: Adjusting for Age				Sensitivity Analysis P-Values		
	ASD Mean (SE)	Control Mean (SE)	ASD vs. Control Difference (95% CI)	P-value	Adjusting for Age and BMI Z-Score	Adjusting for Age and Log-Transformed OPAQ Mets	Adjusting for Age and Log-Transformed Calcium Intake
Distal Radius	N=11	N=17					
Total Area (mm ²)*	339.5 (14.9)	351.2 (20.1)					
Log-Transformed Total Area (mm ²)	5.8 (0.05)	5.8 (0.05)	0.8% (-12.4%, 16.0%)	0.91	0.67	0.12	0.71
Cortical Area (mm ²)*	19.0 (6.05)	22.3 (2.91)					
Log-Transformed Cortical Area (mm ²)	1.88 (0.43)	2.8 (0.19)	-60.6% (-84.5%, 0.5%)	0.051	0.057	0.092	0.076
Trabecular Area (mm ²)*	297.2 (15.2)	310.0 (20.5)					
Log-Transformed Trabecular Area (mm ²)	5.7 (0.05)	5.7 (0.06)	0.8% (-14.3%, 18.5%)	0.92	0.73	0.19	0.75
Percent Cortical Area (%)	5.4 (1.78)	7.1 (1.03)	-1.6 (-5.8, 2.5)	0.44	0.33	0.42	0.44
Cortical Thickness (mm)*	0.24 (0.07)	0.30 (0.04)					
Log-Transformed Cortical Thickness (mm)	-2.5 (0.44)	-1.5 (0.22)	-61.3% (-85.3%, 1.8%)	0.054	0.071	0.078	0.076
Cortical Porosity (%)	0.049 (0.008)	0.046 (0.004)	0.003 (-0.014, 0.020)	0.73	0.56	0.39	0.38
Total Volumetric BMD (mgHA/cm ³)	222.8 (14.4)	239.3 (7.4)	-16.6 (-48.5, 15.4)	0.31	0.31	0.21	0.50
Cortical Volumetric BMD (mgHA/cm ³)	639.1 (23.8)	680.9 (14.4)	-41.8 (-98.1, 14.5)	0.15	0.075	0.14	0.043
Trabecular Volumetric BMD (mgHA/cm ³)	175.5 (8.8)	186.2 (5.6)	-10.7 (-30.9, 9.4)	0.30	0.44	0.14	0.72
Number of Trabeculae (1/mm)	2.3 (0.06)	2.2 (0.05)	0.1 (-0.1, 0.3)	0.28	0.63	0.58	0.35
Trabecular Separation (mm)	0.37 (0.01)	0.39 (0.01)	-0.02 (-0.05, 0.02)	0.33	0.61	0.71	0.33
Trabecular Thickness (mm)	0.063 (0.002)	0.070 (0.002)	-0.007 (-0.012, -0.002)	0.004	0.071	0.011	0.044
Compressive Stiffness (kN/mm)	56.7 (5.07)	69.7 (3.28)	-13.0 (-24.8, -1.3)	0.030	0.062	0.097	0.12
Failure Load (kN)	3.0 (0.25)	3.7 (0.16)	-0.6 (-1.2, -0.1)	0.031	0.054	0.11	0.12
Distal Tibia	N=16	N=18					
Total Area (mm ²)*	767.0 (34.2)	878.6 (68.3)					
Log-Transformed Total Area (mm ²)	6.6 (0.05)	6.7 (0.07)	-8.2% (-22.0%, 8.0%)	0.30	0.17	0.97	0.39
Cortical Area (mm ²)*	72.7 (8.87)	84.6 (5.27)					

Variable	Primary Analysis: Adjusting for Age				Sensitivity Analysis P-Values		
	ASD Mean (SE)	Control Mean (SE)	ASD vs. Control Difference (95% CI)	P-value	Adjusting for Age and BMI Z-Score	Adjusting for Age and Log-Transformed OPAQ Mets	Adjusting for Age and Log-Transformed Calcium Intake
Log-Transformed Cortical Area (mm ²)	4.1 (0.13)	4.4 (0.07)	-23.0% (-42.4%, 3.0%)	0.078	0.040	0.11	0.031
Trabecular Area (mm ²)*	680.1 (35.96)	784.0 (69.54)					
Log-Transformed Trabecular Area (mm ²)	6.5 (0.05)	6.6 (0.08)	-8.1% (-23.8%, 10.8%)	0.37	0.26	0.99	0.50
Percent Cortical Area (%)	9.8 (1.31)	10.7 (0.95)	-0.9 (-4.1, 2.4)	0.59	0.45	0.49	0.38
Cortical Thickness (mm)*	0.68 (0.09)	0.76 (0.003)					
Log-Transformed Cortical Thickness (mm)	-0.57 (0.14)	-0.35 (0.09)	-20.0% (-43.0%, 12.2%)	0.20	0.17	0.16	0.085
Cortical Porosity (%)	0.063 (0.006)	0.060 (0.003)	0.003 (-0.010, 0.017)	0.65	0.45	0.59	0.61
Total Volumetric BMD (mgHA/cm ³)	247.9 (11.7)	252.2 (11.2)	-4.3 (-36.3, 27.6)	0.79	0.66	0.66	0.72
Cortical Volumetric BMD (mgHA/cm ³)	737.7 (18.9)	753.2 (9.9)	-15.6 (-59.7, 28.6)	0.49	0.23	0.47	0.28
Trabecular Volumetric BMD (mgHA/cm ³)	186.8 (5.0)	189.4 (8.6)	-2.6 (-21.7, 16.5)	0.79	0.77	0.73	0.94
Number of Trabeculae (1/mm)	2.2 (0.07)	2.2 (0.06)	0.01 (-0.19, 0.20)	0.96	0.54	0.95	0.74
Trabecular Separation (mm)	0.39 (0.01)	0.39 (0.01)	0.00 (-0.04, 0.04)	0.99	0.58	>0.99	0.77
Trabecular Thickness (mm)	0.071 (0.002)	0.073 (0.003)	-0.002 (-0.009, 0.006)	0.69	0.92	0.70	0.61
Compressive Stiffness (kN/mm)	183.2 (8.3)	210.0 (10.6)	-26.7 (-53.3, -0.2)	0.048	0.008	0.19	0.040
Failure Load (kN)	9.4 (0.41)	10.8 (0.54)	-1.4 (-2.7, -0.04)	0.043	0.005	0.20	0.038

* Comparisons between groups reported for natural log transformed data in the subsequent row (log transformation performed to approximate normality)

Associations between bone microarchitecture and participant characteristics within each study group

Table 4.

Dependent Variable	Independent Variable	Association in ASD group		Association in Control group		Interaction P-Value
		Effect of a 1-unit increase	P-value	Effect of a 1-unit increase	P-value	
Compressive Stiffness at the Radius (N/mm)	Percent body fat (%)	269.8 (−846.9, 1386.6)	0.64	−86.5 (−1118.1, 945.2)	0.87	0.65
	Lean mass (kg)	8900.1 (2757.3, 15042.8)	0.005	11891.0 (9064.4, 14717.6)	< 0.001	0.38
	Estradiol (pg/mL)	965.6 (−1054.1, 2985.4)	0.35	816.7 (−51.3, 1684.7)	0.065	0.89
	Total Testosterone (ng/dL)	32.5 (−26.6, 91.6)	0.28	37.3 (1.6, 72.9)	0.040	0.89
	IGF-1 Z-Score	−9603.0 (−47211.0, 28005.0)	0.62	14568.5 (−2049.9, 31186.8)	0.086	0.25
	Log-Transformed Calcium intake from food (Effect of a 1% increase in mg/day)	27.1 (−173.3, 227.4)	0.79	281.4 (54.5, 508.2)	0.015	0.100
	Log-Transformed Calcium intake from food and supplements (Effect of a 1% increase in mg/day)	20.4 (−177.9, 218.7)	0.84	266.2 (35.7, 496.8)	0.024	0.11
	Log-Transformed Vitamin D intake from food (Effect of a 1% increase in mcg/day)	98.0 (−38.7, 234.7)	0.16	33.0 (−57.0, 122.9)	0.47	0.44
	Log-Transformed Vitamin D intake from food and supplements (Effect of a 1% increase in mcg/day)	49.3 (−91.0, 189.6)	0.49	11.9 (−80.1, 104.0)	0.80	0.66
	Log-Transformed Oxford Physical Activity Questionnaire (Effect of a 1% increase in mets)	72.6 (−59.0, 204.3)	0.28	10.3 (−73.7, 94.3)	0.81	0.43
Failure Load at the Radius (N)	Percent body fat (%)	10.9 (−44.1, 65.9)	0.70	1.7 (−46.7, 50.2)	0.94	0.81
	Lean mass (kg)	456.0 (156.4, 755.6)	0.003	571.1 (419.3, 722.9)	< 0.001	0.50
	Estradiol (pg/mL)	49.0 (−47.0, 145.1)	0.32	43.7 (3.0, 84.3)	0.035	0.92
	Total Testosterone (ng/dL)	1.7 (−1.2, 4.6)	0.24	2.0 (0.2, 3.7)	0.031	0.89
	IGF-1 Z-Score	−462.3 (−2314.6, 1389.9)	0.62	718.6 (−76.6, 1513.8)	0.077	0.25
	Log-Transformed Calcium intake from food (Effect of a 1% increase in mg/day)	1.8 (−8.1, 11.8)	0.72	13.3 (1.6, 25.0)	0.026	0.14
	Log-Transformed Calcium intake from food and supplements (Effect of a 1% increase in mg/day)	1.5 (−8.3, 11.3)	0.77	12.5 (0.7, 24.3)	0.038	0.16
	Log-Transformed Vitamin D intake from food (Effect of a 1% increase in mcg/day)	5.3 (−1.4, 12.0)	0.12	1.3 (−3.2, 5.7)	0.58	0.33

Dependent Variable	Independent Variable	Association in ASD group		Association in Control group		Interaction P-Value
		Effect of a 1-unit increase	P-value	Effect of a 1-unit increase	P-value	
Compressive Stiffness at the Tibia (N/mm)	Log-Transformed Vitamin D intake from food and supplements (Effect of a 1% increase in mcg/day)	2.7 (-4.2, 9.6)	0.44	0.2 (-4.3, 4.8)	0.93	0.56
	Log-Transformed Oxford Physical Activity Questionnaire (Effect of a 1% increase in mets)	3.5 (-3.0, 9.9)	0.29	1.2 (-2.9, 5.2)	0.57	0.55
	Percent body fat (%)	1558.0 (-327.0, 3442.9)	0.11	2423.6 (86.9, 4760.3)	0.042	0.57
	Lean mass (kg)	15617.4 (5854.0, 25380.8)	0.002	24332.5 (9341.3, 39323.7)	0.001	0.34
	Estradiol (pg/mL)	3457.0 (1520.0, 5394.1)	<0.001	3013.2 (755.8, 5270.6)	0.009	0.77
	Total Testosterone (ng/dL)	120.1 (37.1, 203.0)	0.005	128.8 (28.3, 229.3)	0.012	0.90
	IGF-1 Z-Score	-22479.6 (-79948.0, 34988.9)	0.44	41279.5 (-6704.0, 89263.0)	0.092	0.095
	Log-Transformed Calcium intake from food (Effect of a 1% increase in mg/day)	-83.2 (-442.5, 276.1)	0.65	581.3 (132.0, 1030.6)	0.011	0.024
	Log-Transformed Calcium intake from food and supplements (Effect of a 1% increase in mg/day)	-87.7 (-443.3, 267.8)	0.63	521.5 (51.7, 991.4)	0.030	0.043
	Log-Transformed Vitamin D intake from food (Effect of a 1% increase in mcg/day)	152.9 (-142.3, 448.2)	0.31	18.0 (-261.2, 297.1)	0.90	0.52
	Log-Transformed Vitamin D intake from food and supplements (Effect of a 1% increase in mcg/day)	106.9 (-170.7, 384.5)	0.45	-28.6 (-287.6, 230.3)	0.83	0.48
	Log-Transformed Oxford Physical Activity Questionnaire (Effect of a 1% increase in mets)	21.2 (-256.6, 299.0)	0.88	287.5 (93.9, 481.0)	0.004	0.12
Failure Load at the Tibia (N)	Percent body fat (%)	73.0 (-19.7, 165.7)	0.12	137.7 (35.3, 240.0)	0.008	0.36
	Lean mass (kg)	735.5 (266.3, 1204.6)	0.002	1163.4 (374.7, 1952.0)	0.004	0.36
	Estradiol (pg/mL)	166.3 (71.4, 261.2)	<0.001	160.9 (54.6, 267.1)	0.003	0.94
	Total Testosterone (ng/dL)	5.8 (1.8, 9.8)	0.004	6.7 (1.7, 11.8)	0.009	0.78
	IGF-1 Z-Score	-1167.2 (-3905.8, 1571.4)	0.40	2186.3 (-237.1, 4609.7)	0.077	0.072
	Log-Transformed Calcium intake from food (Effect of a 1% increase in mg/day)	-2.7 (-20.2, 14.7)	0.76	27.5 (3.7, 51.3)	0.024	0.045
	Log-Transformed Calcium intake from food and supplements (Effect of a 1% increase in mg/day)	-3.0 (-20.3, 14.3)	0.74	24.5 (0.1, 49.0)	0.049	0.072
	Log-Transformed Vitamin D intake from food (Effect of a 1% increase in mcg/day)	7.9 (-6.3, 22.1)	0.27	-0.7 (-14.7, 13.3)	0.93	0.40

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Dependent Variable	Independent Variable	Association in ASD group		Association in Control group		Interaction P-Value
		Effect of a 1-unit increase	P-value	Effect of a 1-unit increase	P-value	
	Log-Transformed Vitamin D intake from food and supplements (Effect of a 1% increase in mcg/day)	5.2 (-8.2, 18.7)	0.45	-3.0 (-16.0, 10.1)	0.66	0.39
	Log-Transformed Oxford Physical Activity Questionnaire (Effect of a 1% increase in mets)	0.8 (-12.2, 13.8)	0.91	16.6 (6.4, 26.9)	0.001	0.054