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Bone Accrual in Males with Autism Spectrum Disorder

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Abstract

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.

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

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autism spectrum disorder (ASD) compared with typically developing peripubertal age-matched male controls.³ Other groups also have reported low BMD *z*-scores⁴ 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 absorption¹¹; co-occurring gastrointestinal diseases, such as colitis¹²; chronic use of medication such as antiepileptics,^{13,14} antipsychotics,^{15,16} proton pump inhibitors,¹⁷ and selective serotonin reuptake inhibitors¹⁶; hypotonia¹⁸; prematurity¹⁹; 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 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 children²¹ 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) of 9.8%–10.2% and a limit of detection or sensitivity of 2 µg/dL. N-telopeptide was measured by enzyme immunoassay (Wampole Laboratories, Princeton, New Jersey), with an intra-assay CV of 6.9% and a limit of detection or sensitivity of 3.2 nM bone collagen equivalents per liter. Carboxy-terminal collagen crosslinks were analyzed by enzyme-linked immunosorbent assay (Serum CrossLaps ELISA; Immunodiagnostic Systems, Fountain Hills, Arizona), which has an intra-assay CV of 5.2%–6.8% and a limit of detection or sensitivity of 0.02 ng/mL. Salivary cortisol samples were collected at 8 a.m. and 11 p.m. and processed by high-sensitivity enzyme immunoassay (catalog no. 1–3002; Salimetrics, State College, Pennsylvania), with an intra-assay CV of 4%–7% and a limit of detection or sensitivity of 0.007 µg/dL.

Eleven subjects who participated at study initiation were informed of low BMD or any abnormal findings and were recommended to receive vitamin D and calcium supplementation as necessary.

Statistical Analyses

Data were analyzed using SAS version 9.4 (SAS Institute, Cary, North Carolina). The data were assessed for normality, and transformations were performed to approximate normality when required. Linear and logistic mixed-effects models were applied to estimate differences between the ASD and control groups with subject- and family-level random effects to account for correlation between repeated measures for a given subject and among siblings. Results from analyses of continuous data are reported as model-estimated means and SEs or differences of means and 95% CIs; results from analyses of categorical data are reported as model-estimated proportions or ORs and 95% CIs. Multivariate analyses were used to control for baseline physical activity, BMI z-scores, percent body fat, serum vitamin D, and vitamin D intake separately. To account for the effects of season in analysis of 25(OH)D levels, we adjusted for the sine of day of year $\times \pi/365$.³¹ A 2-tailed *P* value < .05,

unadjusted for multiple comparisons, was considered to indicate statistical significance, reflecting our focus on exploratory rather than confirmatory analysis.

Results

Clinical characteristics of all participants at baseline are presented in Table II. These data include 2011 data from previous study participants and 2015 data from newly enrolled participants. Comparison data of returnees and nonreturnees from 2011 is found in Table I. Among the entire cohort ($n = 49$), participants with ASD were younger than controls by approximately 10 months, although this difference was not statistically significant. The ASD group had significantly higher percent body fat (27% vs 20% in controls; $P = .034$) and was significantly less physically active according to the Youth Physical Activity Survey (16% very active vs 74% very active in controls; $P = .013$). Vitamin D intake from food was also lower in the ASD group (5.1 $\mu\text{g/day}$ vs 9.3 $\mu\text{g/day}$; $P = .026$). The ASD and control groups did not differ significantly in terms of bone age, height or BMI z -score, pubertal stage, calcium intake, and serum levels of calcium, phosphorus, 25(OH)D, testosterone, and IGF-1 at baseline or follow-up. As expected, both the ASD and controls groups exhibited significant increases in bone age, pubertal status, lean and fat mass, and levels of gonadal steroids over time (Table III; available at www.jpeds.com).

Bone Density Measures

Eleven of 25 participants in the ASD group (44%) had at least 1 site of low BMD (Hologic z -score < -2.0) at 1 or both visits. No control participants met these criteria. Table IV compares Hologic BMD z -scores between the ASD and control groups at baseline, follow-up, and in terms of change over time. In a cross-sectional comparison using all 49 participants, Hologic BMD z -scores were significantly lower in the ASD group than in the control group at the femoral neck and lumbar spine, and marginally lower at the total hip, with estimated differences ranging from 0.75 to 0.95 SD (Figure 1 and Table V; Table V available at www.jpeds.com). Furthermore, the boys with ASD had lower BMD z -scores compared with controls consistently across all ages (Figure 2; available at www.jpeds.com). Among the 25 returnees, each of these BMD deficiencies in the ASD group compared with controls was also observed at the follow-up visit, with no difference in the rate of bone accrual over time (Table IV). Individual trajectories of BMD z -scores can be seen in Figure 2.

Among 36 participants who had BMD z -scores of the whole body and whole body less the head assessed at the follow-up visit (not assessed at baseline), both measures were approximately 1 SD lower in the ASD group compared with the control group (Table V).

The results of sensitivity analyses are presented in Table V. The results were largely unchanged when these analyses were applied to BMD z -scores and height-adjusted BMD z -scores obtained from the BMD-CS.²⁸ In particular, mean height-adjusted BMD z -scores were lower in the ASD group across all sites at both baseline and follow-up, with estimated differences ranging from 0.61 to 1.11 SD, although differences at the total hip and lumbar spine did not achieve statistical significance. Again, no differences in rates of bone accrual were observed. Similar results were also obtained after adjusting for baseline BMI z -scores,

percent body fat, serum vitamin D level, and vitamin D intake separately. After controlling for baseline physical activity level (ie, very active vs active, low active, or sedentary), many cross-sectional differences in BMD were no longer significant.

Discussion

Our data demonstrate that bone accrual rates in children with ASD do not differ from those in controls during puberty. However, the children with ASD did not catch up, and their BMD *z*-scores remained significantly lower compared with those of controls at all anatomic sites measured. It is encouraging that boys with ASD do accrue bone at the same rate as controls during this developmental interval. Whole body and whole body less head BMD *z*-scores were significantly lower in pubertal children with ASD compared with age-, race-, and sex-specific norms and concurrently enrolled control participants. The International Society of Clinical Densitometry recommends assessment of spine and whole body (or whole body less the head) in children, because these measures best predict fracture risk.²¹ Indeed, we previously reported an elevated risk of hip fracture in both children and adults with ASD compared with controls using a nationwide emergency department database.⁶

These findings suggest that bone deficits occur early in life in children with ASD. In contrast, pubertal bone accrual is not reduced in children with ASD, perhaps reflecting that levels of IGF-1 and gonadal steroids, important factors contributing to pubertal bone accrual,³² did not differ between the groups. As a bone trophic factor, IGF-1 increases bone formation during puberty, and testosterone and estradiol lead to decreased bone resorption (and testosterone also may have bone anabolic effects).³² IGF-1 levels increase during puberty and peak in late puberty,³³ but IGF-1 *z*-scores (which compare levels against the mean for age/pubertal stage) remain constant. The absence of any change in IGF-1 *z*-scores over time in both our ASD and control groups suggests that IGF-1 levels increase appropriately during puberty in children with ASD. Both groups exhibited increasing levels of gonadal steroids (testosterone and estradiol) over the 4-year study period, as was expected and consistent with normal pubertal development. The greater increases in estradiol levels in the ASD group may be a consequence of the higher percent body fat in this group, because aromatase in fatty tissue converts testosterone to estradiol.

Although BMI *z*-scores are an important determinant of BMD,³⁴ BMI *z*-scores did not differ between the 2 groups. Moreover, adjustment for BMI *z*-scores did not change our findings. Given that dual-energy X-ray absorptiometry measures areal BMD, which underestimates BMD in short individuals and overestimates BMD in tall individuals, we performed height adjustments using the longitudinal BMD-CS database, and again our findings did not change.

There was no significant difference between the 2 groups in serum 25(OH)D, calcium, or phosphorus levels, arguing against vitamin D deficiency as a contributor to low BMD in children with ASD. Furthermore, adjustment for vitamin D intake or serum 25(OH)D did not change our findings regarding differences in BMD between the 2 groups. In a previous study, we found a significant difference between groups in vitamin D intake from food,³ as in the present study; however, in the earlier study we also found significant differences in

serum 25(OH)D. Thus, the implication of this finding is unclear, given that in the present study, serum 25(OH)D levels did not differ significantly between the ASD and control groups.

An important finding of the present study is that physical activity levels of returnees were significantly lower in the ASD group compared with the control group, with a much lower proportion of children with ASD reporting high levels of physical activity at baseline and follow-up. After controlling for baseline physical activity levels, we no longer found a difference in BMD between the 2 groups, suggesting that differences in physical activity may help explain the lower BMD in the ASD group. Physical activity is an important determinant of BMD, because mechanical loading of bone leads to an increased bone formation rate by stimulating osteoblastic activity.³⁵ Physical activity is particularly relevant in prepubertal and early pubertal children³⁶; in a meta-analysis assessing the impact of weight-bearing activity on bone, a significant positive effect was found only in prepubertal children.³⁷ This age dependence likely reflects the overriding influence of IGF-1 and gonadal steroids on bone during puberty, and suggests that interventions involving increased physical activity or mechanical loading of bone in children with ASD should start in the early childhood years.

One factor that we did not assess in this study is the extent of hypotonia in our sample of children with ASD. The pull of muscle on bone is known to be bone anabolic, and decreased muscle tone reduces muscle loading on bone and is often associated with reduced muscle strength, a known causative factor in low BMD.^{35,38–40} The coordinated action of muscles on bone ensures appropriate loading of bones and joints. Between 40% and 60% of children with ASD have hypotonia,¹⁸ which may contribute to early bone deficits. Further studies are needed to examine the effect of muscle tone on BMD in children with ASD. Discoordination in children with ASD should be addressed early to ensure that these children will engage in physical activity.

Limitations of this study include its small sample size, the enrollment of only males with ASD, and the use of questionnaires to assess food intake and physical activity without intervening assessment of food and exercise between the 2 time points. Furthermore, boys who were started on anticonvulsant or antipsychotic medication after the baseline visit were not excluded from the follow-up study. Despite the small sample size, however, we detected significant differences in BMD measures that persisted at follow-up, but we may have missed other differences owing to low power. It will be important to conduct larger studies that also include females, particularly given that women are at greater risk than men for bone loss and fractures later in life. We acknowledge that assessment of bone accrual at the hip is less accurate in growing children owing to changes that occur during development in bony landmarks at this site, but nonetheless we report these findings with the assumption that potential inaccuracies are distributed similarly in the ASD and control groups. We previously reported a higher prevalence of hip fractures in children and adults with ASD compared with controls,⁶ and International Society for Clinical Densitometry guidelines indicate that sites other than the spine and whole body may be assessed when necessary based on clinical need.²¹ Importantly, cross-sectional comparisons at baseline and follow-up indicated lower BMD measures in the children with ASD, particularly at the femoral neck.

Healthcare professionals caring for children with ASD may consider screening boys with ASD who have multiple risk factors for low BMD and fractures, including a history of previous fracture, marked hypotonia, low physical activity level, and severe vitamin D deficiency. Early diagnosis of low BMD may allow implementation of such therapeutic measures as physiotherapy, physical activity regimens, and optimal calcium and vitamin D supplementation.

In conclusion, our findings indicate that low BMD measures in children with ASD persisted over the 4-year followup period. Pubertal bone accrual rates did not differ in the children with ASD compared with controls, however, suggesting that low BMD is a consequence of factors present in the prepubertal years. Low levels of physical activity may be an important determinant of bone health in children with ASD.

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Glossary

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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Figure 1.

Cross-sectional BMD *z*-scores at the spine, femoral neck, and whole body less the head in boys with ASD vs controls. For the lumbar spine and femoral neck, BMD *z*-scores from participants' baseline visits (2011 where applicable; 2015 for newly enrolled participants) are summarized. For the whole body less the head, BMD *z*-scores from the 2015 visit are summarized. BMD *z*-scores at all sites were significantly lower in boys with ASD compared with controls.

Figure 2.

BMD z-scores at the spine, femoral neck, and whole body less the head by age in boys with ASD vs controls. Individual trajectories from baseline to follow-up are represented by colored lines. An ordinary least squares regression line is presented in black. The boys with ASD had lower BMD z-scores compared with controls consistently across all ages.

Table 1

Clinical characteristics of returnees and nonreturnees from 2011

Variables	Nonreturnees (n = 11)		Returnees (n = 25)		Nonreturnees vs returnees	
	n	Mean (SE) or % (95% CI)	n	Mean (SE) or % (95% CI)	Difference (95% CI) or OR (95% CI)	P value
Age, y	11	11.0 (0.48)	25	11.0 (0.32)	0.04 (−1.57 to 1.65)	.95
Caucasian race	11	63.8 (21.4–92.0)	24	95.8 (56.2–99.8)	OR, 0.1 (0.0–2.4)	.11
Bone age, y	11	11.4 (0.67)	25	11.3 (0.47)	0.04 (−2.23 to 2.30)	.97
Bone age minus chronological age, y	11	0.36 (0.35)	25	0.28 (0.23)	0.08 (−1.07 to 1.24)	.85
Tanner stage III or greater	11	27.3 (5.4–71.3)	25	24.0 (7.9–54.0)	OR, 1.2 (0.1–11.8)	.85
Height z-score	11	0.50 (0.30)	25	0.43 (0.21)	0.06 (−0.94 to 1.07)	.87
BMI z-score	11	0.15 (0.30)	25	−0.09 (0.20)	0.24 (−0.77 to 1.25)	.55
Lean dry mass, kg	11	6.9 (0.31)	25	6.9 (0.20)	0.07 (−0.96 to 1.09)	.86
Fat mass, kg	10	10.5 (2.16)	25	10.5 (1.46)	0.05 (−7.18 to 7.28)	.99
Percent body fat	10	24.7 (3.04)	25	22.8 (2.03)	1.96 (−8.20 to 12.12)	.62
Serum Vitamin D (ng/mL) (adjusting for seasonality)	10	23.1 (2.19)	25	27.6 (1.43)	−4.6 (−12.8, 3.6)	.17
Vitamin D insufficiency (levels <32 ng/mL) (adjusting for seasonality)	10	92.9 (22.4, 99.8)	25	69.6 (26.9, 93.5)	OR, 5.7 (0.1, 346.1)	.27
Vitamin D deficiency (levels <20 ng/mL) (adjusting for seasonality)	10	24.2 (2.0, 83.2)	25	16.1 (2.9, 55.6)	OR, 1.7 (0.1, 48.1)	.66
Vitamin D intake from food, µg/d	11	6.3 (1.61)	25	7.5 (1.16)	−1.18 (−6.70 to 4.33)	.58
Vitamin D intake from food and supplements, µg/d	11	7.2 (2.55)	25	12.0 (1.85)	−4.8 (−13.6 to 3.9)	.20
% meeting EAR for vitamin D intake	11	35.8 (7.1–80.4)	25	51.9 (22.0–80.4)	OR, 0.5 (0.0–5.7)	.49
Serum calcium, mg/dL	11	9.6 (0.09)	24	9.7 (0.06)	−0.02 (−0.33 to 0.30)	.88
Calcium intake from food, mg/d	11	1095.4 (153.81)	25	1041.8 (111.06)	53.6 (−473.1 to 580.3)	.79
Calcium intake from food and supplements, mg/d	11	1125.7 (156.45)	25	1110.5 (112.97)	15.2 (−520.6 to 551.0)	.94
% meeting EAR for calcium intake	11	54.7 (14.8–89.4)	25	49.7 (20.6–79.0)	OR, 1.2 (0.1–12.9)	.82
Phosphorous, mg/dL	11	4.5 (0.17)	25	4.7 (0.11)	−0.25 (−0.81 to 0.30)	.28
IGF-1 z-score	10	−0.23 (0.29)	25	−0.38 (0.19)	0.15 (−0.83 to 1.12)	.70
Total testosterone, ng/dL	10	190.5 (50.36)	25	106.8 (31.85)	83.7 (−81.7 to 249.2)	.23
Log-transformed total testosterone	10	4.2 (0.53)	25	3.6 (0.33)	0.57 (−1.17 to 2.31)	.42
Estradiol, pg/mL	10	11.6 (2.41)	25	6.4 (1.52)	5.2 (−2.7 to 13.1)	.14

EAR, estimated average requirement.

Table II

Clinical characteristics of participants with ASD and controls at baseline (all participants)

Variables	ASD (n = 25)			Controls (n = 24)			ASD vs controls	
	n	Mean (SE) or % (95% CI)		n	Mean (SE) or % (95% CI)		Difference (95% CI) or OR (95% CI)	P value
Age, y	25	10.9 (0.36)		24	11.7 (0.38)		-0.82 (-2.10 to 0.45)	.17
Caucasian race	25	92.1 (64.0–98.7)		23	82.1 (50.6–95.3)		OR, 2.6 (0.2–28.4)	.37
Bone age, y	25	11.4 (0.47)		24	12.3 (0.51)		-0.95 (-2.63 to 0.73)	.22
Bone age minus chronological age, y	25	0.53 (0.24)		24	0.67 (0.26)		-0.14 (-0.98 to 0.71)	.70
Tanner stage III or greater	25	24.1 (9.0–50.4)		24	37.4 (17.1–63.3)		OR, 0.5 (0.1–2.6)	.36
Height z-score	25	0.44 (0.21)		24	0.84 (0.23)		-0.41 (-1.15 to 0.33)	.23
BMI z-score	25	0.22 (0.20)		24	-0.11 (0.20)		0.33 (-0.35 to 1.02)	.28
Lean dry mass, kg	25	6.6 (0.21)		24	7.4 (0.23)		-0.71 (-1.47 to 0.06)	.064
Fat mass, kg	24	12.7 (1.57)		24	9.7 (1.58)		3.0 (-2.4 to 8.5)	.22
Percent body fat	24	27.3 (1.96)		24	19.7 (1.98)		7.6 (0.8 to 14.4)	.034
Serum vitamin D, ng/mL (adjusting for seasonality)	24	26.7 (1.42)		24	26.7 (1.68)		-0.03 (-5.27, 5.22)	.99
Vitamin D insufficiency (<32 ng/mL) (adjusting for seasonality)	24	75.6 (40.1, 93.5)		24	75.6 (34.2, 94.9)		OR, 1.0 (0.1, 7.8)	>.99
Vitamin D deficiency (<20 ng/mL) (adjusting for seasonality)	24	16.9 (4.2, 48.4)		24	12.0 (1.7, 51.9)		OR, 1.5 (0.1, 20.3)	.71
Vitamin D intake from food, µg/d	25	5.1 (0.99)		24	9.3 (1.01)		-4.2 (-7.7 to -0.7)	.026
Vitamin D intake from food and supplements, µg/d	25	8.8 (1.56)		24	12.6 (1.59)		-3.8 (-9.3 to 1.6)	.14
% meeting EAR for vitamin D intake	25	36.3 (16.2–62.7)		24	62.7 (35.5–83.7)		OR, 0.3 (0.1–1.6)	.14
Serum calcium, mg/dL	24	9.7 (0.06)		24	9.6 (0.06)		0.03 (-0.20 to 0.25)	.79
Calcium intake from food, mg/d	25	952.2 (105.57)		24	1248.4 (109.24)		-296.2 (-667.5 to 75.0)	.099
Calcium intake from food and supplements, mg/d	25	1035.1 (106.55)		24	1269.8 (108.74)		-234.7 (-607.3 to 137.8)	.17
% meeting EAR for calcium intake	25	39.4 (17.7–66.3)		24	65.0 (36.3–85.8)		OR, 0.4 (0.1–1.8)	.16
Phosphorous, mg/dL	25	4.6 (0.11)		24	4.6 (0.12)		-0.07 (-0.47 to 0.33)	.67
IGF-1 z-score	24	-0.15 (0.17)		24	-0.50 (0.19)		0.34 (-0.26 to 0.95)	.21
Total testosterone, ng/dL	24	93.2 (35.78)		24	209.4 (37.42)		-116.1 (-242.1 to 9.8)	.065
Log-transformed total testosterone	24	3.5 (0.35)		24	4.3 (0.35)		-0.76 (-1.96 to 0.45)	.18
Estradiol, pg/mL	24	7.2 (1.89)		24	12.2 (1.96)		-5.0 (-11.7 to 1.6)	.11
Youth physical activity level: very active	25	15.7 (4.3–43.4)		24	74.0 (44.4–91.1)		OR, 0.1 (0.0–0.4)	.013
Oxford Physical Activity Questionnaire METS	19	2480.9 (885.7–4076.1)		18	4256.4 (2617.6–5895.3)		-1775.5 (-4062.6 to 511.5)	.11

Variables	ASD (n = 25)		Controls (n = 24)		ASD vs controls	
	n	Mean (SE) or % (95% CI)	n	Mean (SE) or % (95% CI)	Difference (95% CI) or OR (95% CI)	P value
Log-transformed Oxford Physical Activity Questionnaire METS	19	7.4 (6.8–7.9)	18	8.1 (7.6–8.7)	–0.79 (–1.56 to –0.03)	.044

EAR, estimated average requirement; METS, metabolic equivalents.

Significant P values are in bold type.

Table III

Clinical characteristics of returning participants with ASD and controls at baseline and follow-up, and changes over time

Variables	ASD (n = 14)				Controls (n = 11)			
	Baseline,		Change over time		Follow-up,		Change over time	
	n	mean (SE) or % (95% CI)	Difference (95% CI) or OR (95% CI)	P value	n	mean (SE) or % (95% CI)	Difference (95% CI) or OR (95% CI)	P value
Age, y	14	10.8 (0.44)	3.9 (3.7–4.1)	<.001	11	11.2 (0.51)	4.0 (3.8–4.2)	<.001
Bone age, y	14	10.9 (0.56)	4.6 (3.8–5.5)	<.001	11	11.6 (0.63)	3.7 (2.8–4.7)	<.001
Bone age minus chronological age, y	14	0.14 (0.31)	0.71 (–0.19 to 1.62)	.12	11	0.45 (0.35)	–0.27 (–1.30 to 0.75)	.59
Tanner stage III or greater	14	21.4 (6.3–52.5)	OR, 47.7 (4.2–535.2)	.003	10	30.0 (8.9–65.4)	OR, 21.0 (1.8–251.5)	.018
Height z-score	14	0.26 (0.29)	–0.13 (–0.55 to 0.30)	.55	11	0.68 (0.34)	0.07 (–0.41 to 0.55)	.77
BMI z-score	14	0.09 (0.29)	0.05 (–0.32 to 0.41)	.79	11	–0.31 (0.34)	0.10 (–0.32 to 0.51)	.64
Lean dry mass, kg	14	6.6 (0.26)	2.1 (1.7–2.5)	<.001	11	7.2 (0.30)	2.3 (1.8–2.7)	<.001
Fat mass, kg	14	11.7 (2.07)	6.9 (2.6–11.1)	.003	11	8.9 (2.43)	5.8 (1.0–10.6)	.019
Percent body fat	14	25.3 (2.65)	2.7 (–1.5 to 6.9)	.19	11	19.7 (3.11)	2.9 (–1.8 to 7.5)	.22
Serum Vitamin D (ng/mL) (adjusting for seasonality)	13	25.9 (1.75)	–0.26 (–9.04, 8.51)	.95	11	28.9 (2.01)	–0.17 (–9.83, 9.50)	.97
Vitamin D deficiency (levels <20 ng/mL) (adjusting for seasonality)	13	6.0 (0.3, 61.6)	OR, 1.0 (0.2, 5.5)	.97	11	0.5 (0.0, 33.1)	OR, 10.5 (0.1, 1772)	.35
Vitamin D intake from food (µg/d)	12	5.7 (1.41)	1.82 (–2.32 to 5.96)	.37	11	10.2 (1.50)	–1.32 (–5.65 to 3.00)	.53
Vitamin D intake from food and supplements, µg/d	12	10.5 (2.44)	1.28 (–5.96 to 8.53)	.72	11	15.6 (2.61)	–6.7 (–14.3 to 0.9)	.081
Serum calcium, mg/dL	12	9.7 (0.09)	0.01 (–0.20 to 0.21)	.93	11	9.6 (0.10)	0.03 (–0.19 to 0.24)	.80
Calcium intake from food, mg/d	12	1058.4 (147.38)	51.4 (–320.2 to 423.0)	.78	11	1087.7 (158.57)	477.3 (89.2–865.4)	.018
Calcium intake from food and supplements, mg/d	12	1151.7 (147.44)	–24.5 (–426.2 to 377.1)	.90	11	1140.9 (159.02)	472.3 (52.7–891.8)	.029
Phosphorous, mg/dL	13	4.7 (0.17)	–0.56 (–1.05 to –0.07)	.026	11	4.7 (0.20)	–0.35 (–0.88 to 0.18)	.18
IGF-1 z-score	13	–0.17 (0.31)	–0.07 (–0.84 to 0.70)	.85	11	–0.56 (0.34)	–0.12 (–0.96 to 0.72)	.77
Total testosterone, ng/dL	13	91.4 (37.39)	304.8 (194.9–414.6)	<.001	11	133.9 (40.65)	280.6 (161.2–400.1)	<.001
Log-transformed total testosterone	13	3.6 (0.46)	2.3 (1.5–3.2)	<.001	11	3.8 (0.50)	1.94 (0.99–2.89)	<.001
Estradiol, pg/mL	13	6.0 (1.41)	15.8 (12.5–19.1)	<.001	11	7.1 (1.53)	10.1 (6.5–13.7)	<.001
Youth physical activity level: very active	12	4.5 (0.5–29.7)	OR, 2.6 (0.2–28.0)	.42	11	88.5 (50.8–98.3)	OR, 0.5 (0.1–3.9)	.48

Significant *P* values are in bold type.

Table IV
Areal BMD z-scores (using the Hologic database) of returning participants with ASD and controls

Variables	Timepoint	ASD (n = 14)			Controls (n = 11)			ASD vs controls	
		z-score <-2, % (n)	Mean (SE) or accrual (95% CI)	P value	z-score <-2, % (n)	Mean (SE) or accrual (95% CI)	P value	Difference (95% CI)	P value
Femoral neck	Baseline	21.4 (3)	-1.53 (0.30)		0 (0)	-0.77 (0.32)		-0.76 (-1.33 to -0.18)	.012
BMD z-score	Follow-up	28.6 (4)	-1.41 (0.26)		0 (0)	-0.44 (0.27)		-0.97 (-1.34 to -0.61)	<.001
	Change over time		0.12 (-0.19 to 0.43)	.43		0.34 (-0.01 to 0.68)	.058	-0.21 (-0.68 to 0.25)	.35
Lumbar spine	Baseline	28.6 (4)	-1.18 (0.34)		0 (0)	-0.33 (0.39)		-0.86 (-1.79 to 0.08)	.070
BMD z-score	Follow-up	28.6 (4)	-1.14 (0.32)		0 (0)	-0.08 (0.36)		-1.06 (-1.91 to -0.21)	.016
	Change over time		0.04 (-0.33 to 0.42)	.81		0.25 (-0.18 to 0.68)	.25	-0.20 (-0.78 to 0.37)	.47

Significant *P* values are in bold type.

Table V
Sensitivity analyses of areal BMD (absolute BMD and z-scores) using the Hologic database and the database of the longitudinal BMD-CS

Site	BMD measure	Unadjusted/ adjusted	Population	Timepoint	ASD			Controls			ASD vs controls		
					n	Mean (SE) or accrual (95% CI)	P value	n	Mean (SE) or accrual (95% CI)	P value	Difference (95% CI)	P value	
Femoral neck	BMD, g/cm ²	Unadjusted	Total cohort (n = 49)	Baseline	25	0.61 (0.02)		24	0.73 (0.02)		−0.12 (−0.19 to −0.05)		
			Returnees (n = 25)	Baseline	14	0.60 (0.02)		11	0.71 (0.03)		−0.10 (−0.18 to −0.03)		
				Follow-up	14	0.72 (0.04)		11	0.88 (0.04)		−0.16 (−0.27 to −0.05)		
				Change over time	14	0.11 (0.07–0.15)		11	0.17 (0.12–0.21)		−0.06 (−0.12 to 0.01)		
Hologic BMD z-score	Unadjusted	Total cohort (n = 49)	Baseline	25	−1.54 (0.19)		24	−0.59 (0.20)		−0.95 (−1.60 to −0.29)	.012		
		Total cohort (n = 49)	Baseline	25	−1.64 (0.19)		24	−0.93 (0.29)		−0.72 (−1.51 to 0.08)	.068		
		Returnees (n = 25)	Baseline	14	−1.75 (0.26)		11	−1.29 (0.51)		−0.46 (−1.52 to 0.60)	.38		
			Follow-up	14	−1.63 (0.29)		11	−0.96 (0.52)		−0.67 (−1.79 to 0.44)	.23		
	Adjusting for baseline BMI z-score		Change over time	14	0.12 (−0.19 to 0.43)	.43	11	0.34 (−0.01 to 0.68)	.058	−0.21 (−0.68 to 0.25)	.35		
		Total cohort (n = 49)	Baseline	25	−1.60 (0.18)		24	−0.55 (0.20)		−1.05 (−1.72 to −0.37)	.010		
		Returnees (n = 25)	Baseline	14	−1.66 (0.25)		11	−0.61 (0.30)		−1.04 (−1.83 to −0.26)	.011		
			Follow-up	14	−1.53 (0.29)		11	−0.28 (0.35)		−1.26 (−2.18 to −0.33)	.010		
			Change over time	14	0.12 (−0.19 to 0.43)	.43	11	0.34 (−0.01 to 0.68)	.058	−0.21 (−0.68 to 0.25)	.35		
		Total cohort (n = 48)	Baseline	25	−1.55 (0.19)		24	−0.56 (0.21)		−0.99 (−1.70 to −0.29)	.015		
		Returnees (n = 25)	Baseline	14	−1.53 (0.27)		11	−0.76 (0.32)		−0.77 (−1.48 to −0.05)	.036		
			Follow-up	14	−1.41 (0.28)		11	−0.43 (0.32)		−0.98 (−1.71 to −0.25)	.010		
			Change over time	14	0.12 (−0.19 to 0.43)	.43	11	0.34 (−0.01 to 0.68)	.058	−0.21 (−0.68 to 0.25)	.35		
Adjusting for baseline serum vitamin D (centered at 32 ng/mL)	Total cohort (n = 48)	Baseline	25	−1.54 (0.21)		24	−0.62 (0.22)		−0.93 (−1.63 to −0.22)	.019			
	Returnees (n = 25)	Baseline	14	−1.51 (0.32)		11	−0.77 (0.33)		−0.74 (−1.33 to −0.15)	.016			
		Follow-up	14	−1.39 (0.29)		11	−0.43 (0.30)		−0.95 (−1.37 to −0.54)	<.001			
		Change over time	14	0.12 (−0.19 to 0.43)	.43	11	0.34 (−0.01 to 0.68)	.058	−0.21 (−0.68 to 0.25)	.35			
Adjusting for baseline vitamin D intake (centered at 10 µg/d)	Total cohort (n = 49)	Baseline	25	−1.54 (0.19)		24	−0.58 (0.21)		−0.95 (−1.66 to −0.24)	.018			
	Returnees (n = 25)	Baseline	14	−1.55 (0.31)		11	−0.80 (0.32)		−0.75 (−1.33 to −0.17)	.013			
		Follow-up	14	−1.43 (0.27)		11	−0.46 (0.28)		−0.96 (−1.35 to −0.58)	< .001			
		Change over time	14	0.12 (−0.19 to 0.43)	.43	11	0.34 (−0.01 to 0.68)	.058	−0.21 (−0.68 to 0.25)	.35			
Unadjusted	Total cohort (n = 48)	Baseline	25	−1.41 (0.20)		23	−0.44 (0.23)		−0.97 (−1.68 to −0.26)	.016			
	Returnees (n = 24)	Baseline	14	−1.37 (0.37)		10	−0.47 (0.39)		−0.90 (−1.59 to −0.21)	.013			
BMD-CS z-score [*]													

Site	BMD measure	Unadjusted/ adjusted	Population	Timepoint	ASD			Controls			ASD vs controls	
					n	Mean (SE) or accrual (95% CI)	P value	n	Mean (SE) or accrual (95% CI)	P value	Difference (95% CI)	P value
Total hip	Height-adjusted BMD-CS z-score *	Unadjusted	Total cohort (n = 48) Returnees (n = 24)	Follow-up	14	-1.41 (0.31)		10	-0.31 (0.31)		-1.10 (-1.36 to -0.84)	<.001
				Change over time	14	-0.04 (-0.42 to 0.34)	.84	10	0.16 (-0.29 to 0.62)	.46	-0.20 (-0.80 to 0.39)	.49
				Baseline	25	-1.57 (0.18)		23	-0.63 (0.21)		-0.95 (-1.60 to -0.29)	.012
				Baseline	14	-1.55 (0.27)		10	-0.60 (0.33)		-0.96 (-1.77 to -0.15)	.022
				Follow-up	14	-1.51 (0.29)		10	-0.40 (0.35)		-1.11 (-1.98 to -0.24)	.014
				Change over time	14	0.04 (-0.34 to 0.42)	.84	10	0.19 (-0.26 to 0.64)	.39	-0.16 (-0.75, 0.44)	.59
	BMD, g/cm ²	Unadjusted	Total cohort (n = 49) Returnees (n = 25)	Baseline	25	0.73 (0.02)		24	0.84 (0.02)		-0.11 (-0.19 to -0.04)	
				Baseline	14	0.71 (0.03)		11	0.79 (0.03)		-0.09 (-0.17 to -0.01)	
				Follow-up	14	0.81 (0.04)		11	0.97 (0.05)		-0.16 (-0.29 to -0.02)	
				Change over time	14	0.10 (0.05-0.15)		11	0.17 (0.12-0.23)		-0.07 (-0.14 to 0.00)	
				Baseline	25	-0.66 (0.22)		24	0.09 (0.25)		-0.75 (-1.54 to 0.04)	.060
				Baseline	25	-0.76 (0.23)		24	-0.24 (0.36)		-0.52 (-1.48 to 0.45)	.23
Hologic BMD z-score	Adjusting for baseline youth physical activity level (very active vs less)	Unadjusted	Total cohort (n = 49) Returnees (n = 25)	Baseline	14	-1.03 (0.27)		11	-1.01 (0.53)		-0.02 (-1.13 to 1.08)	.96
				Follow-up	14	-1.43 (0.31)		11	-1.08 (0.55)		-0.35 (-1.54 to 0.84)	.55
				Change over time	14	-0.40 (-0.71 to -0.09)	.015	11	-0.07 (-0.43 to 0.28)	.67	-0.32 (-0.79 to 0.15)	.17
				Baseline	25	-0.70 (0.23)		24	0.10 (0.25)		-0.80 (-1.64 to 0.04)	.057
				Baseline	14	-0.91 (0.28)		11	-0.07 (0.33)		-0.83 (-1.72 to 0.05)	.063
				Follow-up	14	-1.31 (0.32)		11	-0.15 (0.38)		-1.16 (-2.17 to -0.14)	.027
Adjusting for baseline percent body fat (centered at 25%)	Unadjusted	Total cohort (n = 49) Returnees (n = 25)	Change over time	14	-0.40 (-0.71 to -0.09)	.015	11	-0.07 (-0.43 to 0.28)	.67	-0.32 (-0.79 to 0.15)	.17	
			Baseline	25	-0.81 (0.21)		24	0.11 (0.23)		-0.93 (-1.74 to -0.11)	.033	
			Baseline	14	-0.85 (0.30)		11	-0.22 (0.37)		-0.63 (-1.56 to 0.29)	.17	
			Follow-up	14	-1.25 (0.32)		11	-0.29 (0.39)		-0.96 (-1.94 to 0.03)	.056	
			Change over time	14	-0.40 (-0.71 to -0.09)	.015	11	-0.07 (-0.43 to 0.28)	.67	-0.32 (-0.79 to 0.15)	.17	
			Baseline	25	-0.72 (0.26)		24	0.02 (0.27)		-0.74 (-1.58 to 0.11)	.075	
Adjusting for baseline serum vitamin D (centered at 32 ng/mL)	Unadjusted	Total cohort (n = 48) Returnees (n = 25)	Baseline	14	-0.64 (0.36)		11	-0.20 (0.38)		-0.44 (-1.17 to 0.30)	.23	
			Baseline	14	-1.04 (0.34)		11	-0.28 (0.35)		-0.76 (-1.39 to -0.13)	.020	
			Follow-up	14	-0.40 (-0.71 to -0.09)	.015	11	-0.07 (-0.43 to 0.28)	.67	-0.32 (-0.79 to 0.15)	.17	
			Change over time	14	-0.40 (-0.71 to -0.09)	.015	11	-0.07 (-0.43 to 0.28)	.67	-0.32 (-0.79 to 0.15)	.17	

Site	BMD measure	Unadjusted/ adjusted	Population	Timepoint	ASD			Controls			ASD vs controls		
					n	Mean (SE) or accrual (95% CI)	P value	n	Mean (SE) or accrual (95% CI)	P value	Difference (95% CI)	P value	
Change over time		Adjusting for baseline vitamin D intake (centered at 10 µg/d)	Total cohort (n = 49)	Baseline	25	-0.66 (0.23)		24	0.10 (0.25)		-0.77 (-1.62 to 0.09)	.069	
			Returnees (n = 25)	Baseline	14	-0.84 (0.31)		11	-0.23 (0.38)		-0.61 (-1.52 to 0.29)	.18	
				Follow-up	14	-1.24 (0.32)		11	-0.30 (0.39)		-0.94 (-1.88 to 0.01)	.052	
				Change over time	14	-0.40 (-0.71 to -0.09)	.015	11	-0.07 (-0.43 to 0.28)	.67	-0.32 (-0.79 to 0.15)	.17	
	BMD-CS z-score *	Unadjusted	Total cohort (n = 48)	Baseline	25	-0.50 (0.23)		23	0.19 (0.26)		-0.70 (-1.52 to 0.13)	.084	
			Returnees (n = 24)	Baseline	14	-0.70 (0.30)		10	0.11 (0.38)		-0.82 (-1.77 to 0.14)	.090	
				Follow-up	14	-1.33 (0.35)		10	-0.20 (0.43)		-1.13 (-2.24 to -0.03)	.045	
				Change over time	14	-0.63 (-0.98 to -0.27)	.001	10	-0.31 (-0.73 to 0.11)	.14	-0.32 (-0.87 to 0.23)	.24	
	Height-adjusted BMD-CS z-score *	Unadjusted	Total cohort (n = 48)	Baseline	25	-0.68 (0.22)		23	-0.07 (0.24)		-0.61 (-1.38 to 0.16)	.10	
			Returnees (n = 24)	Baseline	14	-0.81 (0.28)		10	-0.11 (0.34)		-0.71 (-1.59 to 0.17)	.11	
				Follow-up	14	-1.29 (0.34)		10	-0.36 (0.41)		-0.93 (-2.01 to 0.15)	.088	
				14	-0.48 (-0.84 to -0.11)	.013	10	.24	-0.22 (-0.79 to 0.34)	.43			
Lumbar spine	BMD, g/cm ²	Unadjusted	Total cohort (n = 49)	Baseline	25	0.59 (0.02)		24	0.70 (0.03)		-0.11 (-0.20 to -0.02)		
			Returnees (n = 25)	Baseline	14	0.57 (0.03)		11	0.64 (0.04)		-0.07 (-0.17 to 0.03)		
				Follow-up	14	0.75 (0.05)		11	0.89 (0.06)		-0.14 (-0.29 to 0.01)		
				Change over time	14	0.18 (0.13-0.23)		11	0.25 (0.19-0.31)		-0.07 (-0.15 to 0.01)		
	Hologic BMD z-score	Unadjusted	Total cohort (n = 49)	Baseline	25	-0.89 (0.22)		24	-0.03 (0.24)		-0.86 (-1.64 to -0.09)	.035	
			Total cohort (n = 49)	Baseline	25	-0.91 (0.23)		24	-0.09 (0.35)		-0.82 (-1.77 to 0.13)	.077	
			Returnees (n = 25)	Baseline	14	-1.15 (0.36)		11	-0.24 (0.49)		-0.91 (-1.93 to 0.11)	.077	
				Follow-up	14	-1.10 (0.34)		11	0.01 (0.47)		-1.11 (-2.06 to -0.17)	.023	
	Adjusting for baseline BMI z-score		Total cohort (n = 49)	Change over time	14	0.04 (-0.34 to 0.42)	.81	11	0.25 (-0.18 to 0.68)	.25	-0.20 (-0.78 to 0.37)	.47	
			Returnees (n = 25)	Baseline	25	-0.98 (0.21)		24	0.00 (0.23)		-0.98 (-1.77 to -0.19)	.024	
				Baseline	14	-1.23 (0.31)		11	-0.19 (0.37)		-1.04 (-2.00 to -0.08)	.035	
				Follow-up	14	-1.19 (0.29)		11	0.05 (0.35)		-1.24 (-2.15 to -0.33)	.009	
Adjusting for baseline percent body fat (centered at 25%)		Total cohort (n = 48)	Change over time	14	0.04 (-0.34 to 0.42)	.81	11	0.25 (-0.18 to 0.68)	.25	-0.20 (-0.78 to 0.37)	.47		
		Returnees (n = 25)	Baseline	25	-0.97 (0.23)		24	0.05 (0.25)		-1.02 (-1.87 to -0.17)	.028		
			Baseline	14	-1.19 (0.33)		11	-0.19 (0.39)		-1.00 (-1.93 to -0.07)	.036		

Site	BMD measure	Unadjusted/ adjusted	Population	Timepoint	ASD			Controls			ASD vs controls		
					n	Mean (SE) or accrual (95% CI)	P value	n	Mean (SE) or accrual (95% CI)	P value	Difference (95% CI)	P value	
Whole body	BMD-CS z-score [*]	Unadjusted	Total cohort (n = 48)	Follow-up	14	-1.15 (0.31)		11	0.05 (0.37)		-1.20 (-2.06 to -0.35)	.008	
				Change over time	14	0.04 (-0.34 to 0.42)	.81	11	0.25 (-0.18 to 0.68)	.25	-0.20 (-0.78 to 0.37)	.47	
			Adjusting for baseline serum vitamin D (centered at 32 ng/mL)	Returnees (n = 25)	Baseline	25	-0.98 (0.25)		24	-0.15 (0.26)		-0.83 (-1.64 to -0.02)	.046
					Baseline	14	-1.49 (0.39)		11	-0.46 (0.41)		-1.03 (-2.06 to 0.01)	.051
			Adjusting for baseline vitamin D intake (centered at 10 µg/d)	Total cohort (n = 49)	Follow-up	14	-1.44 (0.35)		11	-0.21 (0.37)		-1.23 (-2.12 to -0.34)	.009
					Change over time	14	0.04 (-0.34 to 0.42)	.81	11	0.25 (-0.18 to 0.68)	.25	-0.20 (-0.78 to 0.37)	.47
		Unadjusted	Total cohort (n = 48)	Baseline	25	-0.89 (0.22)		24	-0.02 (0.25)		-0.87 (-1.71 to -0.03)	.044	
				Baseline	14	-1.20 (0.34)		11	-0.40 (0.40)		-0.80 (-1.74 to 0.14)	.093	
			Returnees (n = 25)	Follow-up	14	-1.15 (0.32)		11	-0.15 (0.38)		-1.00 (-1.87 to -0.13)	.026	
				Change over time	14	0.04 (-0.34 to 0.42)	.81	11	0.25 (-0.18 to 0.68)	.25	-0.20 (-0.78 to 0.37)	.47	
		Height-adjusted BMD-CS z-score [*]	Unadjusted	Total cohort (n = 48)	Baseline	25	-0.81 (0.24)		23	0.05 (0.27)		-0.86 (-1.73 to 0.01)	.052
					Baseline	14	-1.15 (0.38)		10	-0.22 (0.46)		-0.93 (-2.01 to 0.16)	.091
	Returnees (n = 24)			Follow-up	14	-1.25 (0.37)		10	-0.06 (0.44)		-1.19 (-2.22 to -0.17)	.024	
				Change over time	14	-0.10 (-0.49 to 0.29)	.61	10	0.17 (-0.29 to 0.63)	.46	-0.27 (-0.87 to 0.34)	.37	
	Total cohort (n = 48)			Baseline	25	-0.99 (0.21)		23	-0.31 (0.24)		-0.68 (-1.43 to 0.08)	.071	
				Baseline	14	-1.21 (0.32)		10	-0.53 (0.38)		-0.67 (-1.70 to 0.35)	.19	
	BMD, g/cm ²	Unadjusted	Total cohort (n = 48)	Follow-up	14	-1.29 (0.35)		10	-0.43 (0.42)		-0.86 (-1.99 to 0.27)	.13	
				Change over time	14	-0.09 (-0.51 to 0.34)	.68	10	0.10 (-0.40 to 0.60)	.69	-0.18 (-0.84 to 0.47)	.57	
			2015 cohort (n = 36)		18	0.88 (0.02)		18	1.00 (0.03)		-0.12 (-0.21 to -0.03)		
				2015 cohort (n = 36)		18	-1.36 (0.19)		18	-0.44 (0.21)		-0.92 (-1.57 to -0.27)	.013
			Adjusting for baseline youth physical activity level (very active vs less)	2015 cohort (n = 36)		18	-1.45 (0.20)		18	-0.74 (0.29)		-0.71 (-1.49 to 0.07)	.066
	Hologic BMD z-score	Unadjusted	2015 cohort (n = 36)		18	-1.38 (0.20)		18	-0.43 (0.22)		-0.95 (-1.65 to -0.25)	.018	
				2015 cohort (n = 36)		18	-1.37 (0.20)		18	-0.44 (0.22)		-0.93 (-1.65 to -0.20)	.022
			Adjusting for baseline BMI z-score	2015 cohort (n = 36)		18	-1.43 (0.21)		18	-0.48 (0.22)		-0.95 (-1.64 to -0.26)	.017
					2015 cohort (n = 36)		18	-1.36 (0.19)		18	-0.40 (0.22)		-0.95 (-1.64 to -0.27)
			Adjusting for baseline vitamin D intake (centered at 10 µg/d)	2015 cohort (n = 36)		18	-1.67 (0.24)		17	-0.56 (0.28)		-1.11 (-1.97 to -0.25)	.019

Site	BMD measure	Unadjusted/ adjusted	Population	Timepoint	ASD			Controls			ASD vs controls		
					n	Mean (SE) or accrual (95% CI)	P value	n	Mean (SE) or accrual (95% CI)	P value	Difference (95% CI)	P value	
Whole body less head	Height-adjusted BMD-CS z-score *	Unadjusted	2015 cohort (n = 35)		18	-1.90 (0.22)		17	-0.81 (0.25)		-1.08 (-1.83 to -0.34)	.012	
	BMD, g/cm ²	Unadjusted	2015 cohort (n = 36)		18	0.79 (0.03)		18	0.91 (0.03)		-0.12 (-0.21 to -0.02)		
	Hologic BMD z-score	Unadjusted	2015 cohort (n = 36)		18	-1.24 (0.22)		18	-0.45 (0.24)		-0.79 (-1.53 to -0.04)	.041	
		Adjusting for baseline youth physical activity level (very active vs less)	2015 cohort (n = 36)		18	-1.39 (0.21)		18	-0.96 (0.32)		-0.43 (-1.28 to 0.41)	.24	
		Adjusting for baseline BMI z-score	2015 cohort (n = 36)		18	-1.28 (0.22)		18	-0.44 (0.24)		-0.84 (-1.60 to -0.08)	.036	
		Adjusting for baseline percent body fat (centered at 25%)	2015 cohort (n = 36)		18	-1.26 (0.22)		18	-0.43 (0.25)		-0.83 (-1.65 to -0.01)	.048	
		Adjusting for baseline serum vitamin D (centered at 32 ng/mL)	2015 cohort (n = 36)		18	-1.34 (0.23)		18	-0.51 (0.25)		-0.82 (-1.60 to -0.04)	.042	
		Adjusting for baseline vitamin D intake (centered at 10 µg/d)	2015 cohort (n = 36)		18	-1.21 (0.22)		18	-0.40 (0.25)		-0.81 (-1.58 to -0.04)	.042	
	BMD-CS z-score *	Unadjusted	2015 cohort (n = 35)		18	-1.44 (0.27)		17	-0.42 (0.32)		-1.01 (-1.97 to -0.06)	.041	
	Height-adjusted BMD-CS z-score *	Unadjusted	2015 cohort (n = 35)		18	-1.76 (0.22)		17	-0.80 (0.25)		-0.96 (-1.69 to -0.23)	.018	

Significant *P* values are in bold type.

* z-scores were calculated using the database of the longitudinal BMD-CS.