

Clinical Research Article

Cortical Bone Mass is Low in Boys with Klinefelter Syndrome and Improves with Oxandrolone

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Abbreviations: AMH, anti-Müllerian hormone; BHI, Bone Health Index; BMD, bone mineral density; CV, coefficient of variation; DXA, dual-energy x-ray absorptiometry; DXR, digital x-ray radiogrammetry; FSH, follicle-stimulating hormone; KS, Klinefelter syndrome; LH, luteinizing hormone; OX, oxandrolone; PA, physical activity; PL, placebo; pQCT, peripheral quantitative computed tomography; SDS, standard deviation score.

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Abstract

Context: Klinefelter syndrome (KS) is the most common sex aneuploidy in men. Affected males have hypogonadism, and, as a result, face an increased risk for osteoporosis and fractures. Androgen therapy is standard in adolescents and adults with KS but has not been used earlier in childhood.

Objective: To determine the effects of androgen treatment on bone mass in children with KS.

Methods: Randomized, double-blind, placebo-controlled clinical trial of oxandrolone (OX; 0.06 mg/kg daily; n = 38) versus placebo (PL; n = 40) for 2 years in boys with KS (ages 4–12 years). Changes in bone mass were examined by digital x-ray radiogrammetry, which determines the Bone Health Index (BHI) and standard deviation score (SDS).

Results: BHI SDS was similar between groups at baseline (-0.46 ± 1.1 vs -0.34 ± 1.0 OX vs PL, $P > .05$) and higher in the OX group at 2 years (-0.1 ± 1.3 vs -0.53 ± 0.9 , OX vs PL, $P < .01$). At baseline, BHI SDS values of all subjects were not normally distributed with 25.7% of subjects plotted below -1 SDS ($P < .001$), suggesting a deficit in bone mass. In total, 13.5% of subjects had sustained a fracture and their BHI SDS was lower than those with no fractures (-1.6 ± 1.3 vs -0.3 ± 1.0 , $P = .004$).

Conclusion: Bone mass using BHI SDS is reduced in some children with KS and improves with OX. Since these individuals are at risk for osteoporosis, age-appropriate androgen replacement and future studies on bone health in children with KS should be further explored.

Key Words: cortical bone, oxandrolone, Klinefelter syndrome, boys, fractures, bone health index (BHI)

Klinefelter syndrome (KS) is the most common sex chromosomal aneuploidy with an estimated prevalence of 1:650 male births [1, 2]. Gonadal failure, which involves both Leydig and Sertoli cells, is typically evident during adolescence and is the hallmark of the syndrome [1, 2]. The clinical appearance is highly variable and may include neurocognitive and/or behavior impairment [1, 2]. Increased cardiometabolic risk [1, 3] and decreased bone mass [2, 4, 5] have also been described to occur frequently in KS and contribute to the increased mortality and morbidity of the syndrome [6, 7].

Rates of low bone mass as high as 25% to 43% have been reported in adults with KS, although normal bone mass has also been documented by few investigators [4]. An increased risk of vertebral fractures was recently described [5]. Differences in study design, small sample size, and variability in study cohorts may well explain some of these discrepancies [2, 4, 8]. Additional evidence that bone health is impaired comes from epidemiological studies that identify fractures as a cause of increased mortality and morbidity in KS compared with the general population [6, 7]. Understanding the etiology and developing successful management strategies for the treatment of low bone mass in KS is, therefore, highly desirable.

Androgen deficiency is frequently considered the underlying etiology of low bone mass in KS [9]. Testosterone replacement to improve bone mass or prevent bone loss is routinely recommended in hypogonadal individuals. However, the exact effect of hypogonadism and testosterone therapy on the skeletal health of individuals KS is unclear [2, 4, 8]. Positive correlations between bone mass and serum testosterone concentrations have been reported, but not consistently [9-12]. Similarly, testosterone replacement has shown to improve bone mass in some studies, but results vary [9-12]. A recent meta-analysis of 1141 KS men observed a deficit in bone mass and an improvement with testosterone replacement [13]. Others have suggested that early therapy starting in adolescence is important to normalize bone mass, indicating a role of androgens on normal bone accrual in these patients [12, 14]. This finding is particularly important as suboptimal bone accrual may have life-long adverse effects on bone health and rates of fractures [15]. The variability in outcomes among studies is likely related to the differences in design, as randomized controlled studies are missing and the current literature is based on mostly retrospective, cross-sectional reports.

Hypogonadism is almost universal from midpuberty to adulthood in individuals with KS. Whether testicular

function is impaired in early childhood, and already present during the minipuberty of infancy, remains a topic of debate [16]. Furthermore, some investigators have raised the question of androgen deficiency in early life and its association with certain phenotypic characteristics of KS, including neurocognitive deficits, low bone mass, hypotonia, and an adverse cardiometabolic profile [2]. Therefore, there is a great interest to understand whether androgen administration in early life and childhood results in improvements of the various manifestations of KS. Our study is the first randomized controlled trial of oxandrolone (OX), a weak androgen, in prepubertal boys with KS. OX was deemed an appropriate androgen for this age group as it has been shown not to cause virilization at doses of less than 0.06 mg/kg/day that were used in this study [17]. We have previously reported an improvement in visual-motor function, rates of anxiety, and socialization as well as an improvement in body composition and fasting triglycerides with OX administration in this cohort [18, 19]. An increased risk for gonadarche with OX was also observed [20]. Herein, we report the results of this trial on bone mass. We also describe longitudinal changes in bone mass and describe relationships between bone mass and fractures in prepubertal boys with KS.

Materials and Methods

Study Design

This was a randomized, double-blind trial of oral OX versus PL given for 2 years in boys with KS. Details of the study protocol and enrollment flow diagram have previously been published [17-19] and are summarized here. Inclusion criteria were karyotype 47,XXY, 48,XXYY, or 48,XXXYY with <50% mosaicism for a 46,XY cell line in the blood, ages 4- 12.9 years, testicular volume \leq 4 mL, and no treatment with exogenous androgens in the previous year. For this specific manuscript, analysis was restricted to only 47,XXY participants. Exclusion criteria were 46,XX and 47,XYY males and inability to comply with the study.

Subjects were followed every 6 months for 2 years. Medical history including history of fractures, physical examination, fasting morning laboratory evaluation (ie, glucose, lipids, total and free testosterone, luteinizing hormone [LH], follicle-stimulating hormone [FSH]), body composition by electronic skin caliper, and a bone age x-ray of the left hand were obtained at baseline and every 6 months for the duration of the study. Pubertal

development was assessed according to Tanner staging at every visit by an experienced pediatric endocrine clinician. Testicular volume was estimated by palpation using the Prader orchidometer. Testis volume smaller than the smallest measurement on the Prader orchidometer (1 mL) was estimated as 0.5 mL. Gonadarche was defined as testicular volume of either testis ≥ 4 mL. Physical activity (PA) was measured with a validated parental questionnaire [21] that assesses the child's activities on a typical school day in a typical week. Parents were asked how many minutes the child spends on school days on each of a list of specific activities, which includes sports, active games, and individual activities such as walking, running, and skating [21]. Using this methodology, we have previously shown that boys with KS are less physically active than their peers [22].

Dosing of OX started at 0.06 mg/kg/day and was rounded to the nearest 1.25 mg, with a minimum dose of 1.25 mg daily and a maximum dose of 3.75 mg daily. Dosing was weight adjusted at every 6-month study visit and reduced for side effects as previously described [18–20]. The study was approved by the Human Subjects Committee of Thomas Jefferson University. Written informed consent and assent were obtained. The primary outcomes of the trial involved changes in neurocognitive and psychosocial functions with therapy and were previously published [18]. A secondary analysis reported on the effects of OX on cardiometabolic health and age of gonadarche [19, 20]. The focus of this report is on assessment of bone mass by using automated bone age determination and radiogrammetry.

Laboratory Assessments

A fasting, morning blood sample was obtained at all study visits. Total testosterone was measured by high-pressure liquid chromatography tandem mass spectrometry with interassay and intra-assay coefficient of variation (CV) of $<10\%$ (Esoterix Laboratories, Calabasas Hills, CA). Free bioavailable testosterone was determined after sex hormone binding globulin precipitation and measurement of serum albumin (Esoterix Laboratories, Calabasas Hills, CA). LH, FSH, inhibin B, and anti-Müllerian hormone (AMH) were measured simultaneously from frozen serum. Normal ranges by age and pubertal stage were determined from 304 boys 4.0–13.0 years of age without an endocrine or metabolic disorder. The LH and FSH immunoassays (TRFIA AutoDelfia Wallac; PerkinElmer, Courtaboeuf, France) had limit of quantification of 0.024 and 0.033 mIU/mL, respectively, with interassay and intra-assay CV of $<5\%$ for both assays. LH concentrations >0.3 mIU/mL is consistent with gonadarche.

The inhibin B enzyme-linked immunosorbent assay (AnschLabs, Webster, TX) had a limit of quantification of 2.2 pg/mL, interassay CV of 9.7% and 4.8% at a concentration of 43 and 93 pg/mL, respectively, and an intra-assay CV of 4.6% and 2.8% at concentrations of 53 and 96 pg/mL, respectively. The assay had no cross-reactivity with activins or inhibin A. AMH was measured with an enzyme-linked immunosorbent assay (AnschLabs) down to 0.2 pmol/L. The interassay CV was $<9\%$ at concentrations of 7 to 110 pmol/L with an intra-assay CV $<4\%$ above 5 pmol/L. Values below the limit of detection for any hormone were assigned the lower limit of quantification for the assay.

Bone Health Index

Bone Health Index (BHI) (also known as Pediatric Bone Mass Index) was calculated from left hand radiographs (bone age x-ray) using digital x-ray radiogrammetry (DXR) as previously described [23, 24]. In brief, the method is based on the BoneXpert system for automatic determination of bone age (Visiana, Hørsholm, Denmark, www.BoneXpert.com). For the calculation of BHI, regions of interest are set in the shafts, the 3 middle metacarpals are used, and the software calculates the cortex borders [23–25], and the average values for cortical thickness (T), bone width (W) and bone length (L) are obtained. BHI is then calculated as $BHI = \pi T(1 - T/W)/(LW)^{0.33}$ [25]. BHI reference values have been obtained in a group of healthy European children stratified according to sex and bone age [25]. The BHI results of study participants were then compared to these reference values and expressed as a standard deviation score. BHI has a mean relative SD of 7.5% and precision of 1.42% [25].

Statistical Analysis

The Kolmogorov–Smirnov Test for Normality was used to test the distribution of BHI standard deviation score (SDS) values. Group comparisons at baseline were conducted using t-tests for continuous variables and the Mann–Whitney test for nonparametric variables. As previously reported [14], longitudinal assessment of BHI SDS was performed using a mixed model of repeated measures analysis of covariance, with fixed effects of treatment group and 24-month visit, comparing the change from baseline at 24 months in the OX and PL groups and adjusting for baseline differences in values. Univariate regression analyses were performed to identify baseline factors that were independently predictive of BHI and BHI SDS. Multiple linear regression models were used to identify predictors of BHI after adjusting

for several variables. A subgroup analysis compared BHI SDS by fracture history status. $\alpha \leq .05$ was considered to be statistically significant. Analyses were performed using SAS software (9.2, SAS Institute, Inc., Cary, NC) and GraphPad Prism, version 8.0 for Windows (GraphPad Software, La Jolla, CA).

Results

Subject Characteristics

A total of 93 boys were initially enrolled. Karyotypes included 89 subjects with 47,XXY, 2 subjects with 47,XXY/46,XY (low level mosaicism), 1 subject with 48,XXYY and 1 subject with 48,XXXY. This manuscript is restricted only to 47,XXY subjects, a sample that was used to determine rates of low bone mass and relationship to fractures. Of these patients, 45 were randomized to OX and 44 to PL (Table 1). The ethnic composition was 72% Caucasians, 13% African Americans, 13% Hispanic, and 2% Asians. Boys in the OX group were younger ($P = .01$). There were no baseline differences in weight z, height z, BMI z, bone age, BHI SDS, and percent body fat between groups (Table 1). Eleven subjects were lost to follow-up. Data from 78 subjects ($n = 38$ in the OX group and 40 in the PL group) were included in this longitudinal analysis to determine the effect of OX on the bone. Their baseline characteristics were the same between groups except age, which was younger in the OX group (data not shown).

Subjects were prepubertal on enrollment (testicular volume 1 ± 0.65 mL in the OX group vs 1.4 ± 0.9 mL in PL). At the end of the study, testicular volume was 3.7 ± 2 mL in the OX group vs 2.8 ± 2.6 mL in PL. Twenty-two boys in the OX group and 15 boys in the PL group progressed in central puberty during the study. Gonadarche occurred at a younger age in the OX group (9.8 ± 1.5 vs 12.1 ± 1.0 years; OX vs PL; $P < .01$). Furthermore, 5 boys in the OX experienced sexual precocity (ie, onset of gonadarche before 9 years of age) during the course of the study, whereas none in the PL group did (Fisher exact $P = .049$). Their serum LH and testosterone concentrations are summarized at Table 2. For subjects who experienced gonadarche, pubertal development according to testicular volume and serum testosterone concentrations, corresponded to Tanner 2 pubertal development.

Changes in BHI with Oxandrolone

BHI SDS was similar between groups at baseline (-0.46 ± 1.1 vs -0.34 ± 1.0 , OX vs PL, $P > .05$) (Fig. 1 upper panel). At the end of the 24 month study, BHI SDS was higher in the OX group than in PL (-0.1 ± 1.3 vs -0.53 ± 0.9 , OX vs PL, $P = .0002$). Similarly, mean BHI SDS change from baseline was greater in the OX group ($+0.35 \pm 0.7$) than in PL

Table 1. Baseline subject characteristics of oxandrolone treated and placebo groups upon randomization

	Oxandrolone	Placebo
N	45	44
Age (years)	6.9 ± 2.2	$8.3 \pm 2.5^*$
Bone age	6.1 ± 2.5	7.5 ± 2.8
Height Z	0.53 ± 1.0	0.75 ± 1.1
Weight Z	0.55 ± 1.0	0.78 ± 1.0
BMI Z	0.4 ± 1.1	0.6 ± 1.15
BHI SDS	-0.47 ± 1.1	-0.27 ± 1.0
Body fat % SDS	0.98 ± 0.9	1.1 ± 0.8
Physical activity score	46 ± 5.9	45.8 ± 6.5

Oxandrolone treated subjects were younger than the placebo group. there were no other differences between groups. $P = .01$

(-0.2 ± 0.6) ($P < .01$), indicating a positive effect of OX on cortical bone mass.

Biochemical and body composition data at the beginning of the study and changes during the course of the trial are summarized on Table 2. Serum total and free testosterone and serum gonadotropin concentrations rose in a similar fashion in both study groups. No differences between groups were identified at the end of the study. We observed a decrease in % body fat, an increase in lean mass and a decrease in serum triglyceride levels with OX, as previously described [19]. There was no increase in hematocrit with therapy. Changes in BHI SDS during the course of the study did not correlate with changes in % body fat, serum testosterone, gonadotropin, or lipid concentrations (data not shown).

An ad hoc subgroup analysis was performed including only subjects who experienced gonadarche during the course of the trial. Similar changes in BHI SDS were observed with OX ($P < .01$ between the 2 study groups at end of year 2). BHI SDS increased in the OX group from -0.3 ± 0.9 to $+0.2 \pm 0.9$ (baseline vs end of the study, $P < .01$), while no changes were found in the PL group (BHI SDS -0.6 ± 0.5 at baseline vs -0.8 ± 0.5 at 2 years, $P > .05$) (Fig. 1 middle panel). A similar subanalysis that included subjects who remained prepubertal throughout the study failed to document a difference in BHI SDS between groups with therapy, albeit the power of this analysis was low at 0.4. A trend towards a decrease in BHI SDS was observed with PL over the 2 years of the study ($P = .07$), while no such changes were observed with OX (Fig. 1 lower panel).

Cortical Bone Mass in Boys with KS

Data of all subjects at baseline were used to determine deficits in cortical bone mass. Mean BHI SDS of all subjects at baseline was decreased at -0.36 ± 1.1 , indicating a deficit in bone mass ($P = .0014$, 1 sample t test, subjects' BHI

Table 2. Biochemical data for both groups at baseline and end of the study

	Oxandrolone		Placebo	
	Baseline	End of the study	Baseline	End of the study
Testosterone total (ng/dL)	3 (2-5)	22 (3-91) ^a	4 (3-8)	7 (4.5-109) ^a
Testosterone free (pg/mL)	0.2 (0.1-0.5)	4.1 (0.8-19.7) ^a	0.4 (0.2-0.9)	0.6 (0.3-14.3) ^a
LH (mIU/mL)	0.03 (0.01-0.07)	0.5 (0.15-1.7) ^a	0.05 (0.02-2.8)	0.18 (0.03-2.2) ^a
FSH (mIU/mL)	0.5 (0.3-0.7)	0.7 (0.5-1.5)	0.5 (0.3-1.1)	0.4 (0.9-2.2)
AMH (ng/mL)	876 (492-1334)	723 (328-1096)	681 (401-1134)	518 (170-1181)
Inhibin B (pg/mL)	90 (63-143)	107 (79-158)	77 (47-106)	74 (47-112)
TSH (μIU/mL)	2.3 ± 1.1		2.3 ± 1.0	
Free thyroxine (ng/dL)	0.8 ± 0.1		0.8 ± 0.1	
Total cholesterol (mg/dL)	165 ± 33	148 ± 27	162 ± 25	164 ± 33 ^b
HDL (mg/dL)	46 ± 10.4	34 ± 7.3 ^a	44.5 ± 10.1	47.5 ± 12.7 ^{a,b}
LDL (mg/dL)	104 ± 28	102 ± 23	102 ± 23	97 ± 27
Triglycerides (mg/dL)	75.4 ± 58.2	64 ± 52 ^a	68.4 ± 31	82 ± 56.2 ^{a,b}
Fasting blood glucose (mg/dL)	85.0 ± 8.4	87 ± 10.5	85 ± 7	92 ± 11 ^a
Hemoglobin (g/dL)	13.0 ± 0.7	13.4 ± 0.9	13.0 ± 0.8	13.4 ± 0.9

Abbreviations: AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LH, luteinizing hormone; TSH, thyrotropin.

Hormonal results that are non-normally distributed are presented as median and interquartile range.

Normal ranges: total testosterone (mean, range): Tanner I = 4.9 ng/dL (<2.5-10), Tanner II = 42 ng/dL (18-150), Tanner III = 190 ng/dL (100-320), Tanner IV = 372 ng/dL (200-620), Tanner V = 546 ng/dL (350-970). Free testosterone Tanner I = <0.2-3.4 pg/mL, Tanner II = 12 pg/mL (2-58), Tanner III = 30 pg/mL (12-70), Tanner IV and V = 210 pg/mL (84-350).

^a*P* < .05 paired analysis for baseline compared to end of the study within a group.

^b*P* < .05 between groups after adjusting for age and baseline value.

SDS is compared with the theoretical mean BHI SDS = 0 in healthy children). In total, 57.7% of children had a BHI SDS below 0. [Figure 2](#) presents the subject distribution according to their BHI SDS and shows a skewed distribution towards lower values (*P* < .001) with 22% of children plotting between BHI SDS -1 to -2. A total of 25.7% subjects had values below -1. To understand changes in bone mass over time, we determined differences in BHI SDS in the placebo only group. BHI SDS decreased from -0.34 ± 1.0 at baseline to -0.53 ± 0.9 at 24 months, *P* = .05, raising concerns about suboptimal bone accrual.

Data of all subjects at baseline were also used to determine predictors of bone mass. BHI SDS correlated with weight z, BMI z and % body fat z. In a multivariate analysis, weight z was the only predictor of BHI SDS. There was no correlation with serum testosterone concentrations, % body fat, waist circumference, waist to hip ratio, physical activity scores, or total cholesterol or triglycerides. Bone age data were not included in this analysis as BHI SDS is already computed from reference values as a function of bone age.

Fractures

Seven subjects reported a total of 9 fractures upon enrollment. Six additional fractures occurred during the course of the study. By the end of the study, 12 subjects (13.5%) had sustained a total of 15 fractures. Most fractures

(12/15) involved long bones of upper and lower extremities. In a few cases, fractures occurred after falling or being pushed during playing. However, in most cases, history of trauma was not obtained. No difference in fracture numbers between OX and PL groups was observed, granting that the small sample size renders this result hard to interpret. To determine the role of bone mass, we examined differences in BHI SDS at baseline between subjects with fractures compared with those with no fractures. We observed significantly lower BHI SDS in children with fractures vs no fractures: BHI SDS (mean ± SD) = -1.6 ± 1.3 vs -0.32 ± 1.0 , fractures vs no fractures, *P* = .004 ([Fig. 3](#)).

Physical activity scores of all study participants at baseline were compared with previously published healthy controls [22]. Boys with KS were less physically active (PA score: 45.8 ± 6.1 vs 48.1 ± 5.4 , KS subjects vs controls, *P* = .04). We then looked at PA scores among subjects of this study according to their fracture history. There was no difference in physical activity scores between those with fractures (45.78 ± 5.4) and those with no fractures (46.6 ± 6.2). However, boys with fractures spent more time running (running score: 5.3 ± 4.7 vs 10 ± 6.8 , no fractures vs fractures, *P* = .003).

Discussion

This randomized, double blind, placebo-controlled trial in prepubertal boys with KS reports on the effect of OX on

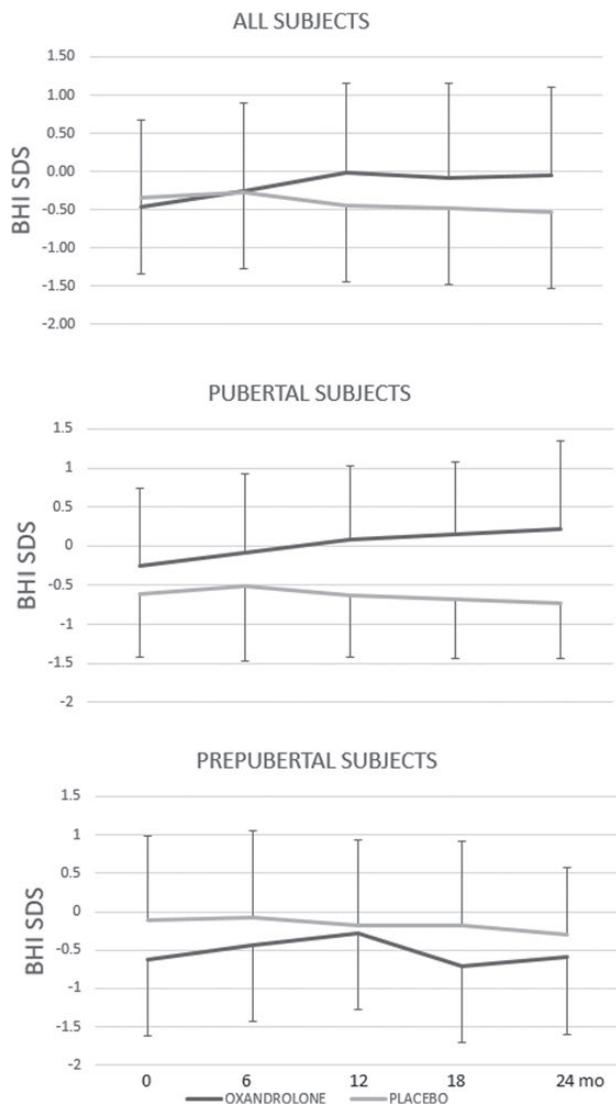


Figure 1. BHI SDS measurements with oxandrolone therapy vs placebo during the course of the 2-year study, showing an improvement with oxandrolone in all subjects (upper panel) and in subjects who reached central puberty during the course of the study (middle panel) ($P < .01$). BHI SDS data from subjects who remained prepubertal are shown at the lower panel: no significant changes with OX were observed. Points are mean \pm SD.

the bone using automated radiogrammetry. We observed a deficit in cortical bone mass at baseline and an improvement with OX therapy. The results support a positive effect of androgens on the skeleton in prepubertal boys with KS.

The seminal role of androgens in bone accrual and bone homeostasis in males is well documented [26-28]. Loss of androgens in men and male rodents leads to loss of bone mass [26]. During puberty, males experience increased periosteal bone formation, and as a result, greater cortical bone acquisition than females [27, 28]. In terms of mechanism, androgens mediate their effects on the skeleton via aromatization to estradiol, while an additional direct effect via the androgen receptor has also been described [26-28].

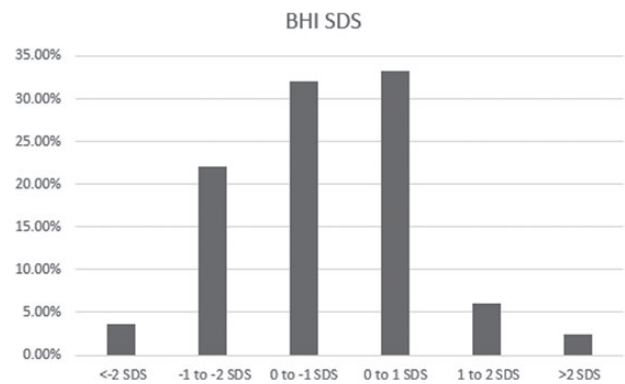


Figure 2. Distribution of study participants according to their BHI SDS at baseline showing a greater proportion of subjects in the BHI SDS range -1 to -2 ($P < .001$).

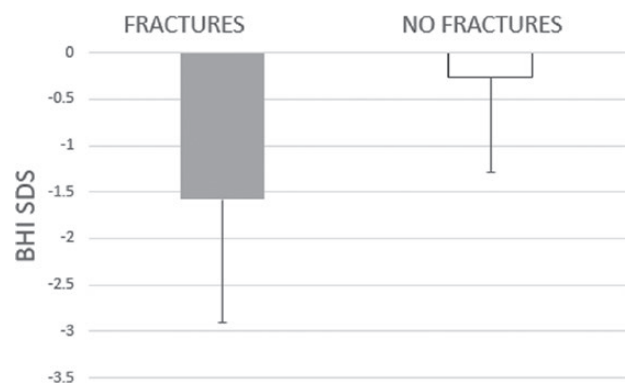


Figure 3. BHI SDS values at baseline in participants with a history of fractures (gray) and participants without fractures (white). Values are expressed as mean \pm SD. $P = .004$.

Furthermore, an androgen-induced increase in muscle mass, which in turn increases the mechanical strain on the skeleton, has been proposed to mediate some of the androgen effects on cortical bone [27]. OX, a synthetic oral nonaromatizable testosterone derivative, has only a weak androgenic activity and low hepatotoxicity. OX binds to androgen receptors in the skeletal muscle to initiate protein synthesis and anabolism, and for these actions OX has been used as an anabolic agent in severely burned children [29, 30]. In severely burned children, OX at 0.1 mg/kg daily for approximately 2 years resulted in a significant improvement of whole body and lumbar Bone mineral content and lumbar bone mineral density (BMD), indicating an effect of OX on both trabecular and cortical bone [30]. In vitro, OX stimulates osteoblast differentiation, most likely through the androgen receptor albeit additional mechanisms have also been proposed [31]. In our study, we were able to document an increase in cortical bone mass with OX in youth with KS, while changes in trabecular bone were not assessed. This result is expected to be independent of estrogen action, as OX is a nonaromatizable

testosterone derivative. However, we have previously reported an earlier onset of gonadarche and adrenarche with OX in some subjects of this cohort [20]. An additional effect on the bone, mediated by the aromatization of pubertal androgens, cannot be excluded. In support of this hypothesis, we observed an increase in bone mass with OX in children who experienced gonadarche during the course of the study, while no such benefits were detected among those who remained prepubertal. However, the power of our observations in the prepubertal subgroup was small, and potential divergent effects of OX according to the presence or absence of gonadarche need to be confirmed with larger studies. Finally, lean mass is known to positively influence bone accrual in children [15] and OX was found to increase muscle mass in severely burned children [29]. It is conceivable that some of the beneficial effects of OX on cortical bone mass that we observed are mediated through an increase in lean mass. Changes in lean mass were not measured in our trial and certainly more studies are needed to clearly delineate the interactions of OX, sex steroids, and body composition on bone mass in children.

Increased bone fragility and low bone mass have been well described in men with KS [2, 4-7]. Whether these facts hold true in prepubertal and peripubertal boys with KS is uncertain. Relevant data are limited to couple of studies that observed differing results on bone mass, while rates of fractures are not reported [32, 33]. This paper provides evidence that bone health is compromised in children with KS. Our study is not large enough to report on the incidence of fractures with confidence. In terms of etiology, fracture rates in children are increased with activity and sports participation [34]. In our study, we did not observe a relationship between fractures and overall physical activity, although boys with fractures spent more time in activities such as running. Children with KS frequently manifest low muscle tone and poor motor coordination [2], factors that may also lead to falls and fractures. Of greater interest, our data suggest that low bone mass is involved in fracture rates, as we were able to document a difference in cortical bone mass between boys with fractures compared to those with no fractures at baseline. In this study, we used BHI as a method to assess bone mass. Using BHI, we observed a deficit in bone mass in prepubertal boys with KS, with approximately 30% of subjects having a measurement below -1 SDS. We also found a decrease in bone mass in the placebo group over the 2-year course of the study, implying that bone accrual is suboptimal. These results bear clinical significance as childhood and adolescence are known, critical time- windows for building optimal peak bone mass, and, thus, may influence life-long rates of osteoporosis and fractures [15]. They are also particularly relevant to individuals with KS, who face high risk for osteoporosis and

fractures as adults, and experience increased mortality as a result of these comorbidities [2, 6]. Although these findings need to be strengthened and validated by further studies, the data raise the importance of optimizing bone mass starting in prepubertal years in KS. Such efforts may include ensuring adequate calcium and vitamin D intake and encouraging physical activities that promote bone health. The latter is particularly important for boys with KS, as they are known to be less physically active than their peers [22]. Based on the outcomes of this study and their significance, we advocate for further research to better understand bone accrual and the impact of androgens on skeletal health in children and adolescents with KS.

The traditional way to assess bone mass is dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) [35]. Automated DXR from bone age x-rays represents an innovative alternative technique that is straightforward to analyze, has low radiation exposure (0.00012 mSv), and does not require special equipment [23-25]. In our specific trial, it was a particularly attractive methodology as bone age x-rays were monitored routinely in an effort to avoid a negative effect of OX on epiphyseal maturation [18]. DXR differs in principle from bone densitometry, since it involves measurements of geometric dimensions (distances) of the bone while densitometry captures mineral densities [25]. DXR is, therefore, insensitive to abnormal mineralization and osteomalacia. It also provides information only on cortical bone and not on trabecular bone [25], which limits our understanding of bone mass changes in KS. Studies in children indicate that cortical bone mass is linked to risk for fractures [34]. Furthermore in a recent case-control study of children who sustained an injury, measurements of cortical bone mass and area were lower in those who suffered a fracture than in those who did not sustain a fracture [36]. Collectively, these data point to the importance of cortical bone in determining risk for fragility and skeletal health in children and support the relevance of our findings in boys with KS. BHI, an index developed using DXR, has the benefit of adjusting for bone length and width, and, thus, corrects for variable body size making it suitable for pediatric studies [25]. The index has been shown to have high degree of precision and a normative dataset that can be used as a reference has been developed [25]. Relevant to this study, BHI SDS is adjusted for bone age. Hence, the differences with OX administration that we observed in this trial are unrelated to bone age changes. DXR results were found to correlate well with BMD measurements by DXA in middle-aged and elderly women [37]. In children, BHI SDS has showed strong correlations with areal and height-adjusted BMD measurements by DXA, including

low DXA values of $z < -2$ [38-42]. Similar correlations have been observed between BHI SDS and total BMD as assessed via pQCT [40]. Although these data support DXR as an alternative methodology for assessment of bone mass in children, larger studies are still needed to validate these findings, describe associations with fractures, and establish the role of DXR in clinical care and research in pediatrics. Considering all factors, the BHI results of this study provide first evidence for below-average bone mass and the benefits of androgen therapy on the bone health in KS that need to be confirmed with additional studies using DXA or pQCT.

Strengths of this work include the prospective, randomized, double-blind design. The sample size was small to determine rates of fractures associated with OX treatment, albeit we were able to demonstrate a statistically significant relationship between fractures and bone mass. Bone mass was assessed using DXR, which does not allow for determination of trabecular bone. Additional studies using alternative methodologies, such as QCT, are needed to determine the effect of androgens on trabecular bone in KS. Although our results are limited to cortical bone, our findings remain significant as cortical bone mass is linked to risk for fragility in children [34, 43]. Data showing strong correlations between BHI SDS and DXA measurements are emerging; however, DXR has not been fully validated against DXA or as a predictor of fractures. The reference data used to calculate BHI SDS is based on Caucasian children [25]. This fact does not largely affect the randomized results of this study. Furthermore, the ethnic distribution of subjects in our study is primarily Caucasian. DXR has been validated in 4 ethnicities, including a Los Angeles pediatric population [44-46]. Although ethnic differences among Caucasian, Asian, and Black children have been described, these variances are small [44-46]. The primary Caucasian constitution of our sample and the validation of DXR in 4 ethnicities mitigate the lack of controls from our site. History of trauma associated with fractures is missing in most of our subjects. Therefore, we cannot determine with certainty that these fractures represent true, low-impact trauma, fragility fractures. Measurements of serum vitamin D concentrations and laboratory evaluation for secondary causes of low bone mass, such as abnormalities of calcium and phosphate excretion, were not performed. Clinically, none of the subjects had manifestations of Cushing's. Markers of bone turnover were not measured, although their validity in pediatric bone health is limited [43]. While we demonstrated that OX has a direct beneficial effect on bone mass in the presence of central puberty, our analyses had insufficient power to detect a similar effect in the absence of puberty. Finally, longer term studies are needed to determine whether OX therapy has an impact on final

height, as more children treated with OX experienced gonadarche at a younger age compared with PL including some cases of true sexual precocity.

In summary, we have shown that OX improves cortical bone in prepubertal boys with KS. We also provide evidence that low bone mass is present in young boys with KS and that bone accrual may be adversely affected. As individuals with KS are vulnerable to low bone mass and fractures, ascertaining optimal bone health starting from childhood becomes important. Our findings support that androgen therapy should be considered in prepubertal and adolescent boys with KS to ensure optimal bone accrual. Further studies employing additional methodologies that assess both trabecular and cortical bone mass and bone density are also needed to fully understand skeletal health, fragility and potential associated factors during childhood and adolescence in KS.

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