

Effects of Oxandrolone on Cardiometabolic Health in Boys With Klinefelter Syndrome: A Randomized Controlled Trial

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Context: Klinefelter syndrome (KS) is a common condition in males, resulting in androgen deficiency and cardiometabolic diseases. These interrelated conditions may be present in prepubertal boys with KS.

Objective: To determine whether supplemental low-dose androgen has a beneficial effect on body composition in prepubertal boys with KS.

Design, Setting, and Participants: We conducted a secondary analysis of a randomized, double-blind, placebo-controlled clinical trial in 93 boys with KS aged 4 to 12 years.

Interventions: Oral oxandrolone (Ox) 0.06 mg/kg/d or placebo for 2 years.

Outcome Measures: The primary outcome was percent body fat standard deviation score (%BF SDS) at 2 years. Secondary outcomes included additional measures of cardiometabolic health and safety.

Results: The %BF SDS at 2 years was significantly lower in the treatment (0.29 ± 0.76 SDS) compared with placebo group (0.81 ± 0.72 SDS) after adjusting for age and baseline %BF SDS (95% confidence interval for the difference between means -0.86 to -0.19 SDS, $P = 0.009$). Ox resulted in lower triglycerides ($P = 0.043$), but also lower high-density lipoprotein (HDL) cholesterol ($P < 0.001$) and a more rapid advancement in bone age ($P = 0.011$).

Conclusions: Ox has positive effects on measures of cardiometabolic health in prepubertal boys with KS; however, it does lower HDL cholesterol and advance bone age. (*J Clin Endocrinol Metab* 102: 176–184, 2017)

One in every 650 males is born with an extra X chromosome, a condition known as Klinefelter syndrome (KS) (1). Despite how common this chromosomal aneuploidy is, historically fewer than 30% of all males with KS were ever diagnosed and only a fraction of these in childhood (2). Recent changes in prenatal genetic screening have increased the number of children known to have KS, and this trend is expected to continue (3, 4). Therefore, the demand is

high for advancing our knowledge of both the natural history and interventions that improve outcomes in this population.

Men with KS have testicular failure with nearly universal infertility as well as hypergonadotropic hypogonadism (5). Testosterone replacement is standard of care in men with KS (6). Testosterone replacement in infants, children, and adolescents with KS is quite variable with a lack of an evidence basis

or generally accepted clinical practice guidelines (5, 7, 8). Due to difficulties in evaluating serum hormone concentrations in prepubertal children, it is not clear whether hypogonadism is present in boys with KS prior to puberty (7). Clinical evidence supporting testosterone deficiency in children with KS includes low-normal stretched penile length with slow penile growth, frequent hypotonia, decreased muscle mass, and poor endurance (9–11). To our knowledge, low-dose androgen supplementation in prepubertal boys with KS has not been evaluated in a randomized design.

Men with KS also have a high morbidity and mortality related to cardiometabolic diseases (12–15). An unfavorable body composition with increased adiposity and decreased muscle mass is common (16, 17). Up to half of men with KS have metabolic syndrome (MetS), a constellation of findings reflecting insulin resistance (16–19). It was recently recognized that even young boys with KS have a high prevalence of MetS features, as well as increased adiposity with average body fat percentage approximately 1 standard deviation above the normal mean (20–22). Furthermore, the risk of MetS features and percent body fat standard deviation score (%BF SDS) in children with KS appear to be independent of age and body mass index (BMI) (21, 23).

Testosterone deficiency in males is known to cause increased adiposity and decreased insulin sensitivity, and treatment with androgens improves these measures (24–26). Given the known testicular dysfunction in KS, hypogonadism is believed to be the underlying etiology of the poor cardiometabolic outcomes in this population (19). We recently found that testicular function is inversely associated with features of MetS in a cross-sectional study of prepubertal boys with KS (23). Therefore, we hypothesized that exogenous androgen treatment would have favorable effects on the cardiometabolic health of boys with KS.

Methods

Overall study design

This was a randomized clinical trial of oral oxandrolone (Ox) *versus* identical-appearing placebo (Pl) in 93 boys with KS. Ox is a nonaromatizable anabolic steroid that is available in an oral tablet in low doses, and is therefore more practical for children than currently available formulations of testosterone (27). Study visits occurred every 6 months for 2 years. The primary outcome for this secondary analysis is %BF SDS at the final 24-month study visit, with the *a priori* hypothesis that Ox would result in lower %BF SDS. Secondary outcomes included additional measures of cardiometabolic function and safety data. Data on the primary outcomes (motor, cognitive, and psychosocial) are being prepared in a separate manuscript.

Setting, recruitment, and participants

This study took place at Thomas Jefferson University (TJU, Philadelphia, PA) from 2007 to 2011. Participants were recruited through internet advertisements, advocacy organizations (predominately Association for X&Y Chromosome Variations, formerly KS&A), and clinical referral. Inclusion criteria were as follows: Karyotype XXY, XXYY, or XXXY with <50% mosaicism for a 46,XY cell line in blood, age 4.0 to 12.9 years of age, testicular volume ≤ 4 mL, and no treatment with exogenous androgens in the previous year. This study was approved by the Human Subjects Committee at TJU and was registered on clinicaltrials.gov (NCT00348946). All participants provided assent, and written informed consent was obtained from parents.

Randomization

Simple randomization using a computer-generated randomization sequence assigned sequentially enrolled participants to 1 of 2 treatment groups (Ox or Pl) with a 1:1 allocation ratio. The allocation sequence was generated by the TJU pharmacy and therefore concealed to the investigators enrolling participants. Study medication was secured and dispensed by the TJU research pharmacy, and all investigators and participants were blinded to the assigned treatment group.

Dosing protocol and safety monitoring

Dosing of the study medication started at 0.06 mg/kg/d 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. The dose was reduced by 50% if any of the following occurred during the study period: low-density lipoprotein (LDL) >159 mg/dL, high-density lipoprotein (HDL) <20 mg/dL, liver enzymes (alanine aminotransferase) exceeding twice the upper limit of normal for the assay (or >90 IU/L), Tanner 2 pubic hair development in boys <8 years of age, bone age advancement >12 mo in a 6-month period and bone age more than chronological age, or systolic and/or diastolic blood pressure (BP) >95th percentile for age, height, and sex. Participants receiving 1.25 mg daily were reduced to 1.25 mg every other day. If a dose reduction occurred due to these predetermined criteria at any point, the subject continued on a 50% dose for the remainder of the trial. The principal investigator maintained the ability to make dose adjustments for additional reasons, such as rapid growth and/or behavioral concerns. An independent Data and Safety Monitoring Board reviewed annual interim analyses on safety measures only; no interim analyses for efficacy were done. The protocol was not changed during the study period, and there were no significant protocol deviations.

Study assessments

Physical examination, body composition, fasting morning blood draw, and bone age x-ray of the left hand occurred at baseline and every 6 months for 2 years (total of 5 complete assessments). Measurements of height, weight, BMI, and waist circumference were converted to SDS using age- and gender-specific norms (28). BP was obtained by auscultation on the arm while in the seated position. Body composition

was assessed by a single trained examiner (K.K.) using the electronic skin fold caliper and calculator Skyndex System I (Skyndex, Albuquerque, NM), which was calibrated before each use. Skin fold measurements were taken from the right triceps and calf, after which the instrument calculated the %BF using the Slaughter/Lohman formula for boys [%BF = 0.735 (triceps + calf) + 1.0] (29). BF percentage was converted to SDS using age- and gender-specific normative data (30). Skin fold measurements are an inexpensive, noninvasive method to assess body composition (31). The Slaughter equation has been shown to accurately estimate %BF compared with dual energy x-ray absorptiometry, particularly in prepubertal, nonobese children like our cohort (32). Bone age of the left hand was interpreted by a single pediatric endocrinologist (J.L.R.) according to the methods of Greulich and Pyle (33).

Fasting blood glucose, LDL, HDL, total cholesterol, and triglycerides were measured using commercial assays. Cutoff values to dichotomize normal from abnormal were defined by the de Ferranti criteria for MetS, as follows: waist circumference >75th percentile for age, triglycerides \geq 100 mg/dL, HDL <50 mg/dL, fasting blood glucose >110 mg/dL, and systolic or diastolic BP >90th percentile for age and height (34). The presence of three or more of these features was considered consistent with a diagnosis of MetS. As there are no universally accepted criteria for MetS in children, we elected to use the same criteria used by other studies of MetS in children with KS (20, 23, 34).

Statistical analyses

The primary analysis was a modified intention to treat analysis; all subjects were analyzed with the group initially randomized to; however, only subjects with data for the primary outcome were included in the final analysis. Missing data were assessed by comparing baseline demographic and outcome variables between completers and noncompleters using a Welch 2-sample *t* test. Additionally, Little's test of Missing Completely at Random was used to test for any missing data mechanisms.

The primary outcome was %BF SDS at 2 years. An ordinary least squares regression model was used with treatment group,

age, and baseline %BF SDS as explanatory variables for the outcome of %BF SDS at 2 years. To explore the time-related pattern of response to treatment, %BF SDS mean and standard error for both groups were plotted across time and compared between treatment groups using Welch's 2-sample *t* test at each time point. Results were considered statistically significant with α of 0.05. A post hoc subanalysis including only participants who remained prepubertal for the entire study (testicular volume always <4 mL, pubic hair Tanner stage 1) was performed due to concerns about the known influence of puberty on cardiometabolic outcomes, particularly insulin resistance.

Secondary outcomes included additional measures of cardiometabolic function and safety measures and were also assessed with multiple linear regression models adjusting for age and baseline measure of the outcome for continuous variables and Fischer exact test for binary outcomes. Results were again graphed across time to appreciate temporal trends. As the secondary analyses were exploratory and measuring similar outcomes, no adjustments were made for multiple comparisons. Statistical analysis was performed using GraphPad Prism, version 6.00 for Mac (GraphPad Software, La Jolla, CA) and R: A Language and Environment for Statistical Computing, version 3.3.1 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 93 boys were recruited and enrolled in the 2-year study. Thirteen subjects withdrew due to lack of interest, and 1 subject had missing data for the primary endpoint; therefore, 79 subjects were included in the analysis for the primary outcome (Fig. 1). There were no differences in baseline characteristics between completers and noncompleters (all *P* values >0.05; data not shown). Additionally, Little's test of Missing Completely at Random was nonsignificant, indicating data were missing at random. There were no more than 3%

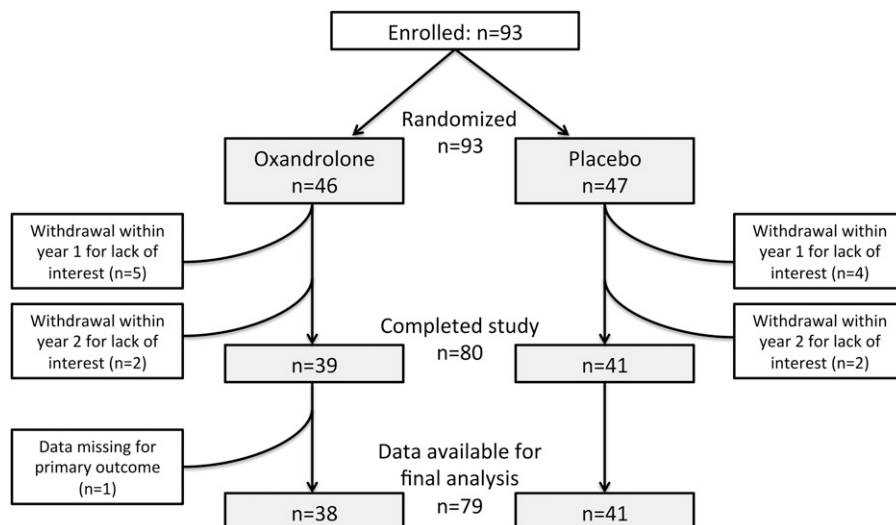


Figure 1. Flow diagram of study participants.

missing data for any variables of interest. Given the lack of differences between completers and noncompleters, a listwise deletion was used for analysis of the cardiometabolic outcomes for a total of 79 subjects.

Baseline demographic and cardiometabolic data were normally distributed and are summarized in Table 1 for all subjects. The Ox group was 1.6 years younger than the Pl group ($P = 0.03$), but the groups were otherwise well matched at baseline. The majority of subjects in both groups were white and diagnosed with 47,XXY prenatally. Common reasons for postnatal testing and diagnosis included developmental delays or behavior concerns. There were no statistical differences in our cardiometabolic endpoints between the pre- and postnatal diagnosed boys.

Body composition

The primary outcome of %BF SDS at 2 years was significantly lower in the Ox group compared with the Pl group (Table 2). This remained significant after adjusting for age and baseline %BF SDS. Reduction in %BF SDS between the beginning and end of the 2-year study was seen in both Ox [-0.61 SDS, 95% confidence interval (CI) -0.86 to -0.36] and Pl groups (-0.40 SDS, 95% CI -0.66 to -0.14). The overall effect size of Ox on %BF

SDS relative to Pl was moderate (Cohen's D 0.71). Visual trends in %BF SDS over time show an increase in the first 6 months of treatment, followed by a decrease in the second year of treatment (Fig. 2).

Boys treated with Ox gained an average of 8.5 ± 3.1 kg lean body mass during the study, which is 33% of their baseline weight, whereas boys in the Pl group gained 7.1 ± 4.4 kg in lean body mass, or 23% of their baseline weight (difference between groups 10%, 95% CI 4.9% to 15%; $P < 0.001$). Waist circumference percentile and BMI SDS were not significantly different between groups.

Additional cardiometabolic measures

Unadjusted comparisons of additional cardiometabolic outcomes at 2 years were significant for lower fasting triglycerides ($P = 0.037$), fasting blood glucose ($P = 0.032$), and systolic BP ($P = 0.022$) in the treatment group. After adjusting for age and baseline cardiometabolic variable, the Ox group had significantly lower fasting triglycerides; however, systolic BP and fasting blood glucose outcomes did not reach statistical significance (Table 2). Total and HDL cholesterol were significantly lower in the Ox group, whereas LDL cholesterol was not different between groups. All but 2 subjects receiving Ox had a decrease in HDL to <50 mg/dL within the first

Table 1. Baseline Demographic and Clinical Characteristics

	Oxandrolone (n = 46)	Placebo (n = 47)
Age in years (range)	6.9 \pm 2.2 (4.0–11.0)	8.5 \pm 2.6 (4.0–12.9)
Karyotype		
47,XXY	45 (98%)	44 (94%)
47,XXY/46,XY	1 (2%)	1 (2%)
48,XXYY or 48,XXXY	0	2 (4%)
Prenatal diagnosis	29 (63%)	28 (60%)
Ethnicity		
White	33 (72%)	34 (72%)
African American	6 (13%)	6 (13%)
Hispanic	5 (13%)	3 (7%)
Asian/Pacific Islander	2 (4%)	3 (6%)
Other	0 (0%)	1 (2%)
Tanner 1 pubic hair	43 (93%)	38 (81%)
BMI SDS	0.40 \pm 1.15	0.58 \pm 1.17
%BF SDS	0.98 \pm 0.92	1.13 \pm 0.84
Waist circumference percentile		
>75th percentile	55 \pm 29.6	61 \pm 30.4
Total cholesterol, mg/dL	167 \pm 33	162 \pm 27
LDL cholesterol, mg/dL	106 \pm 31	104 \pm 24
Triglycerides, mg/dL	75.4 \pm 58.2	67.7 \pm 32.6
≥ 100 mg/dL	9 (20%)	6 (13%)
HDL cholesterol, mg/dL	46.0 \pm 10.4	44.8 \pm 10.1
<50 mg/dL	31 (67%)	31 (66%)
Fasting blood glucose, mg/dL	84.8 \pm 8.4	85.2 \pm 6.7
>110 mg/dL	1 (2%)	0 (0%)
Systolic blood pressure, mm Hg	87.2 \pm 9.7	90.3 \pm 9.4
>90th percentile for age and height	0 (0%)	0 (0%)
Diastolic blood pressure, mm Hg	52.3 \pm 6.9	56.9 \pm 7.3
>90th percentile for age and height	0 (0%)	0 (0%)

Data are mean \pm SD or number (%).

Table 2. Adjusted Cardiometabolic Outcomes at 2 Years by Treatment Group

	Oxandrolone (n = 38)	Placebo (n = 41)	Difference in Means ± SD (95% CI)	<i>p</i> Value ^a
Primary outcome				
%BF SDS, mean ± SD	0.29 ± 0.76	0.81 ± 0.72	−0.52 ± 0.17 (−0.86 to −0.19)	0.009 ^b
Secondary outcomes, mean ± SD				
Total cholesterol, mg/dL	149 ± 28	165 ± 33	−16.0 ± 6.9 (−29 to −2)	<0.001 ^b
LDL cholesterol, mg/dL	101 ± 24	99.6 ± 27	1.45 ± 5.7 (−10.0 to 12.9)	0.813
HDL cholesterol, mg/dL	35.0 ± 7.9	48.5 ± 12.7	−13.5 ± 2.4 (−18.2 to −8.8)	<0.001 ^b
Triglycerides, mg/dL	63.9 ± 51.9	83.5 ± 57.2	−19.6 ± 12.3 (−44.2 to 4.9)	0.043 ^b
Fasting blood glucose, mg/dL	86.6 ± 10.9	91.3 ± 12.2	−5.7 ± 2.6 (−10.9 to −0.5)	0.084
Systolic blood pressure, mm Hg	88.5 ± 11.3	94.2 ± 9.9	−5.7 ± 2.5 (−10.6 to −0.8)	0.164

^a *P* values were derived from ordinary least squares model regressing the outcome at 2 years on treatment group, age, and baseline value of the outcome.

^b *P* value statistically significant at an $\alpha = 0.05$.

6 months of treatment; however, there was no further decline over time (Fig. 3).

Given the universal lowering of HDL, all but 1 boy in the Ox group had at least 1 feature of MetS by the end of the study. Three boys in the Ox group (8%) and 5 boys in the Pl group (12%) met full criteria for MetS (≥ 3 features) at the end of the study. There were no differences between groups in the number of subjects who had an increase or a decrease in the number of MetS features.

Subanalysis of prepubertal boys only

In a post hoc subanalysis, including only boys who remained prepubertal throughout the 2-year study ($n = 43$), results were quite similar to the whole group despite the smaller sample size. Boys treated with Ox ($n = 20$) had a %BF of 0.26 ± 0.73 SDS compared with 0.96 ± 0.80 SDS in boys who received Pl ($n = 23$), after

controlling for age and baseline %BF SDS, $P = 0.009$. Total cholesterol, triglycerides, HDL cholesterol, and systolic BP were all lower in the Ox group ($P = 0.001$, 0.023 , < 0.001 , and 0.008 , respectively). Fasting blood glucose was also lower but did not reach statistical significance ($P = 0.29$).

Safety profile

Overall, Ox was well tolerated and there were no serious adverse events attributable to the drug. No one withdrew for concerns for safety. There were no differences in the reported adverse events between groups (Table 3). In addition to the decline in HDL, the treatment group experienced bone age advancement; however, after 2 years, bone age was only 0.1 years more advanced than chronological age in the treatment group (bone age minus chronological age 0.1 ± 1.1 in Ox, -0.6 ± 1.2 in Pl, 95% CI for the difference in means 0.15 to 1.2, $P = 0.012$; Fig. 3). Height SDS also increased in the treatment group to 0.9 ± 1.2 SDS; therefore, the effect on predicted adult height was minimal. There were no statistically significant differences between groups for liver function or hemoglobin concentration at the end of the study (Fig. 3).

Four subjects (2 Ox, 2 Pl) started the study at a lower dose due to elevated LDL ($n = 2$) or bone age advanced > 1.5 years beyond chronological age ($n = 2$). The study medication dose was lowered by predetermined safety criteria in 41% (19/46) of the Ox group and 26% (12/47) of the Pl group ($\chi^2 P = 0.13$). At the end of the study, the medication dose was 0.042 mg/kg/d in the Ox group and 0.045 mg/kg/d in the Pl group, which were significantly lower than the protocol specified dose of 0.06 mg/kg/d but not different between groups. Reasons for lowering the dose included bone age advancement (11 Ox, 10 Pl), HDL < 20 mg/dL (6 Ox, 0 Pl), and LDL > 159 mg/dL (4 Ox, 2 Pl). No dosing changes were required due to premature pubarche (pubic hair prior to age 8 years), hypertension, or elevated alanine aminotransferase. Both subjects (1 Ox, 1 Pl) who started

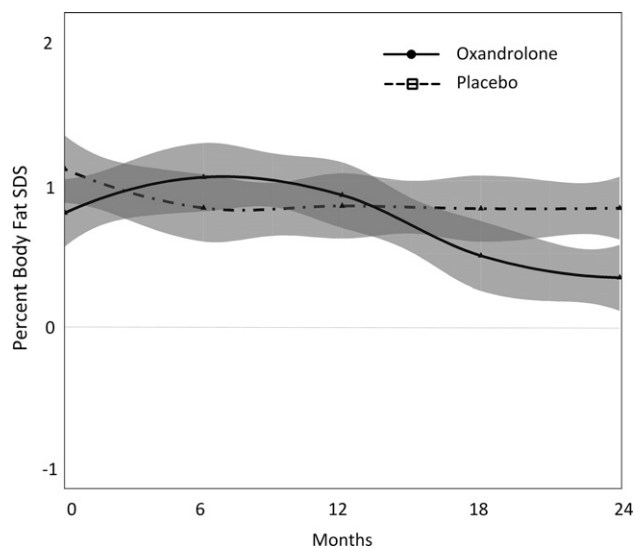


Figure 2. %BF SDS loess curves over the 2-year study period for those treated with Ox (solid line) and placebo (dashed line). The shaded gray curves represent the standard error.

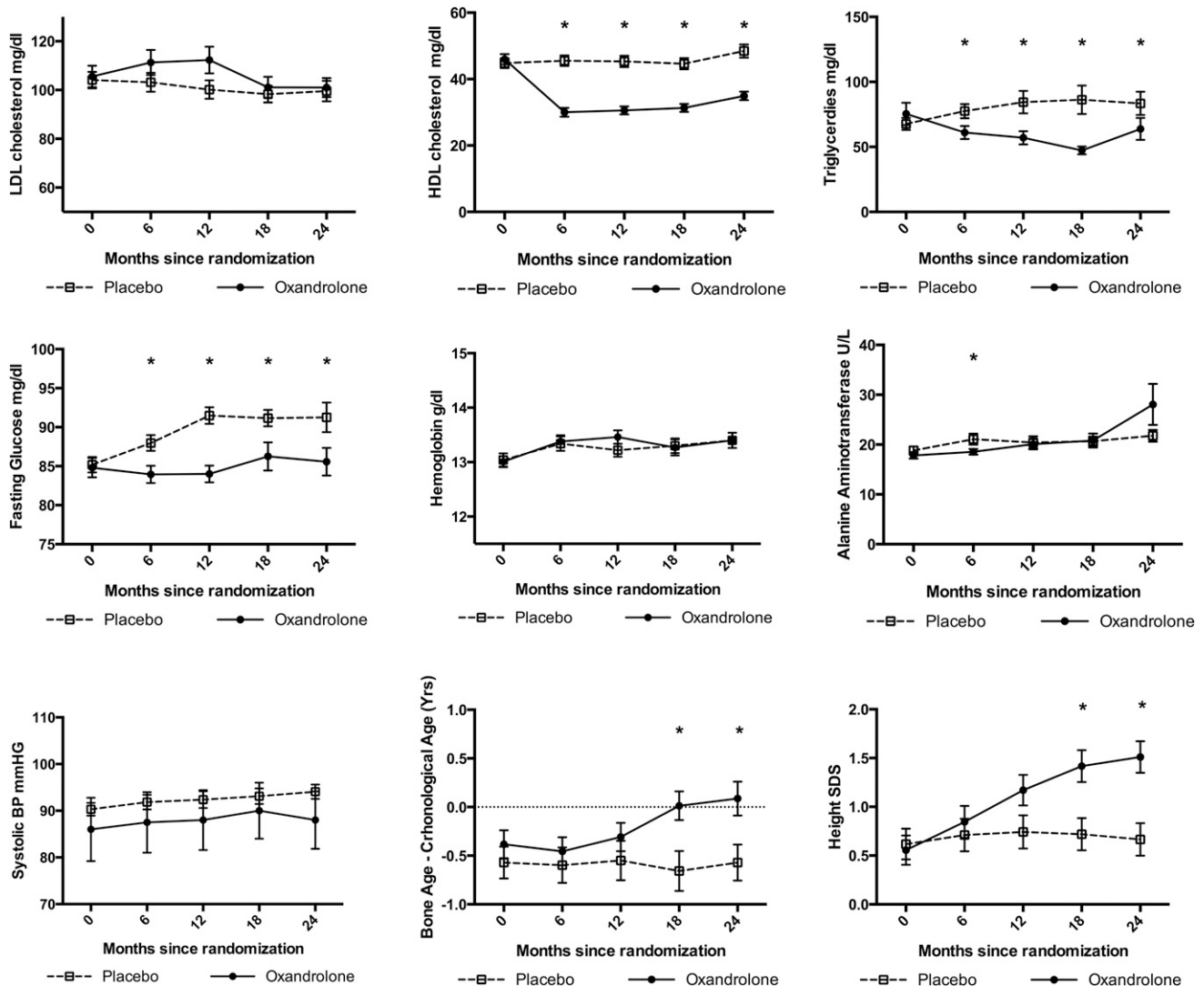


Figure 3. Trends in selected cardiometabolic and safety secondary outcome measures over the 2-year study period for those treated with Ox (solid black line) compared with placebo (dashed). Error bars indicate the standard error. Asterisk represents a significant difference between Ox and placebo groups at that time point with an alpha of 0.05.

with a protocol-specified dose >2.5 mg required dose reductions.

Discussion

In this randomized, double-blind, PI-controlled trial in prepubertal children with KS, 2 years of Ox modestly improved body composition with lowering of %BF. Ox also modestly improved select features of MetS, including lower fasting blood glucose, triglycerides, and BP. Overall, Ox was relatively well tolerated, although it did result in bone age advancement and universal lowering of HDL cholesterol.

Males with KS are recognized to have a high prevalence of cardiometabolic diseases, and these conditions are the leading cause of morbidity and mortality in this population (12–15). Evidence supports these conditions are associated with hypogonadism; however,

testosterone treatment in adults with KS often does not ameliorate the disease state (12). Recent studies, including ours, support that abnormal cardiometabolic markers are already present in boys with KS prior to puberty (20, 21). Therefore, interventions in childhood before the development of disease are more likely to yield preventative benefits to cardiometabolic health in this population. This study evaluated the efficacy of androgen supplementation on markers of cardiometabolic health in prepubertal boys with KS.

Body composition

%BF SDS for the boys in the this study was $+1.1 \pm 0.9$ at baseline, which is very similar to the %BF of $+0.9$ SDS reported by Aksglaede et al. (21) in 18 children with KS assessed by dual energy x-ray absorptiometry. Body composition is an important indicator of cardiometabolic health in children and adults (35, 36), and adiposity strongly

Table 3. Tabulated Adverse Events and/or Parent-Reported Symptoms During the 2-Year Study by Treatment Group

	Oxandrolone (n = 46)		Placebo (n = 47)	
	Total Events	Number of Subjects (%)	Total Events	Number of Subjects (%)
Musculoskeletal complaints	54	29 (63)	53	29 (62)
Infections (any)	77	36 (78)	77	38 (81)
Headache	56	21 (46)	42	22 (47)
Seizure ^a	1	1 (2)	0	0 (0)
Emergency room visits	19	13 (28)	8	7 (15)
Musculoskeletal	5	5 (11)	2	2 (4)
Asthma/allergy	4	3 (7)	0	0 (0)
Gastrointestinal	2	2 (4)	1	1 (2)
Neurologic	2	2 (4)	1	1 (2)
Infection	4	3 (7)	3	3 (6)
Other	1	1 (2)	1	1 (2)
Hospitalizations	2	2 (4)	2	2 (4)
Medical	1	1 (2)	1	1 (2)
Psychiatric	1	1 (2)	1	1 (2)

All *P* values were >0.05 for comparison between groups using Fischer exact test.

^a Preexisting seizure disorder.

correlates with type 2 diabetes in men with KS (17, 19). Androgens, including Ox, are anabolic steroids that positively alter body composition by increasing lean body mass and decreasing adiposity (27, 37). The findings of this blinded study are therefore expected: oral Ox nearly normalized %BF after 2 years, resulting in significantly lower %BF SDS compared with Pl. It is interesting that there was not a decrease in BF until the second year on Ox. Given the trend observed, it is quite possible the benefits of Ox on body composition would become more apparent with longer durations of treatment. It is important to note that the results were most significant in the boys who remained prepubertal. In these younger children, those who were in the Pl group had no change in their %BF, which stayed at about +1 SDS, whereas those who received Ox had a significant decline in their %BF, results that were quite significant despite the reduced sample size. Endogenous or exogenous androgens could have this positive effect on body composition; therefore, the boys who became pubertal were exposed to endogenous androgens and their %BF SDS decreased as a result, even in the Pl group. More research is needed to further understand the trajectory of body composition in both prepubertal and pubertal boys with KS, and ultimately if these observations in childhood track into adulthood.

Features of MetS

Systolic BP, fasting blood glucose, and triglycerides all improved with treatment, although, after adjusting for age and baseline measures, results were only significant for triglycerides. For the prepubertal subgroup, these results were even more significant. Given that up to half of men with KS have MetS (12), it is prudent to evaluate the efficacy of potential treatments on these outcomes. A beneficial effect of Ox was appreciated for several

features of MetS; however, no difference was noted for waist circumference percentile, and HDL cholesterol worsened. Due to these equivocal changes, the number of children who met criteria for MetS did not differ between groups. It is possible that the improvements in these measures with childhood androgen treatment would become more apparent over time and ultimately help prevent the development of these conditions in this population. Our study was also underpowered to detect differences in the number of MetS features over time; therefore, future studies enrolling more children followed for longer durations are needed to draw conclusions about the potential beneficial effect of Ox on MetS features.

Safety profile

Ox was generally well tolerated, as has previously been reported in other childhood populations (27, 38, 39). However, 41% required a dose reduction due to pre-specified criteria, and therefore the final dose was closer to 0.04 mg/kg/dose, and all subjects with a dose >2.5 mg daily required a dose reduction. Bone age advancement and dyslipidemia were the most common reasons for requiring a dose reduction; therefore, if boys with KS are treated with Ox, we would recommend monitoring bone age and lipid panels. Bone age advancement was appreciated despite that Ox is a nonaromatizable androgen that should not convert to estrogen (27). This may be due to stimulation of endogenous testosterone production, as we have previously reported an increase in both serum testosterone and testicular size in boys treated with Ox (40). Given the concurrent increase in height SDS, the predicted adult height is unchanged; therefore, the bone age advancement may not have clinical relevance. The

decrease in HDL cholesterol is a typical response to exogenous androgens (41); therefore, it is not unexpected. This diminution seemed to wane with time; however, the long-term implications of lowering this protective biomarker are unknown. Further investigation is needed to determine whether low HDL is associated with morbidity and mortality in this population and the impact of chronic Ox treatment on HDL cholesterol.

Strengths of this study include the randomized, controlled, and blinded study design with a novel dose-reduction protocol, large number of participants, and good retention over a 2-year study period. As with all studies in this population, there is an ascertainment bias in the individuals who have a prepubertal diagnosis of KS, given the large majority of males with KS are not diagnosed in childhood, and a bias in the families who decide to participate in research. Aside from this unavoidable ascertainment bias, a limitation of this study is our assessment measures of cardiometabolic health do not allow us to understand the physiologic mechanisms of change. Finding differences in these outcomes, however, arguably has more clinical implications than measures of cardiometabolism physiology that are often done for research purposes and supports the need for additional research into underlying mechanisms. Finally, it was not possible to study long-term outcomes, including fertility.

In summary, the result of a 2-year, double-blind, PI-controlled trial of Ox in boys with KS yields modest benefits in some cardiometabolic markers, including % BF SDS and fasting triglycerides; however, Ox notably decreased HDL cholesterol and results in mild bone age advancement. Overall, the short-term cardiometabolic effects of Ox in prepubertal boys with KS are beneficial; however, additional studies are needed to understand the effect of Ox on long-term cardiometabolic health.

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References

- Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab*. 2003;88(2):622–626.
- Abramsky L, Chapple J. 47,XXY (Klinefelter syndrome) and 47, XYY: estimated rates of and indication for postnatal diagnosis with implications for prenatal counselling. *Prenat Diagn*. 1997;17(4): 363–368.
- Practice Bulletin No. 163: Screening for fetal aneuploidy. *Obstet Gynecol*. 2016;127(5):e123–e137.
- Porreco RP, Garite TJ, Maurel K, Marusiak B, Ehrich M, van den Boom D, Deciu C, Bombard A; Obstetrix Collaborative Research Network. Noninvasive prenatal screening for fetal trisomies 21, 18, 13 and the common sex chromosome aneuploidies from maternal blood using massively parallel genomic sequencing of DNA. *Am J Obstet Gynecol*. 2014;211(4):365.e1–365.e12.
- Davis SM, Rogol AD, Ross JL. Testis development and fertility potential in boys with Klinefelter syndrome. *Endocrinol Metab Clin North Am*. 2015;44(4):843–865.
- Radicioni AF, Ferlin A, Balercia G, Pasquali D, Vignozzi L, Maggi M, Foresta C, Lenzi A. Consensus statement on diagnosis and clinical management of Klinefelter syndrome. *J Endocrinol Invest*. 2010;33(11):839–850.
- Fennoy I. Testosterone and the child (0–12 years) with Klinefelter syndrome (47XXY): a review. *Acta Paediatr*. 2011;100(6):846–850.
- Davis S, Howell S, Wilson R, Tanda T, Ross J, Zeitler P, Tartaglia N. Advances in the interdisciplinary care of children with Klinefelter syndrome. *Adv Pediatr*. 2016;63(1):15–46.
- Zinn AR, Ramos P, Elder FF, Kowal K, Samango-Sprouse C, Ross JL. Androgen receptor CAGn repeat length influences phenotype of 47,XXY (Klinefelter) syndrome. *J Clin Endocrinol Metab*. 2005; 90(9):5041–5046.
- Ratcliffe SG, Tierney I, Nshaho J, Smith L, Springbett A, Callan S. The Edinburgh study of growth and development of children with sex chromosome abnormalities. *Birth Defects Orig Artic Ser*. 1982; 18(4):41–60.
- Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF, Zinn A. Early androgen deficiency in infants and young boys with 47, XXY Klinefelter syndrome. *Horm Res*. 2005;64(1):39–45.
- Salzano A, Arcopinto M, Marra AM, Bobbio E, Esposito D, Accardo G, Giallauria F, Bossone E, Vigorito C, Lenzi A, Pasquali D, Isidori AM, Cittadini A. Management of endocrine disease: Klinefelter syndrome, cardiovascular system and thromboembolic disease: review of literature and clinical perspectives. *Eur J Endocrinol*. 2016;175(1):R27–R40.
- Pasquali D, Arcopinto M, Renzullo A, Rotondi M, Accardo G, Salzano A, Esposito D, Saldamarco L, Isidori AM, Marra AM, Ruvolo A, Napoli R, Bossone E, Lenzi A, Baliga RR, Sacca L, Cittadini A. Cardiovascular abnormalities in Klinefelter syndrome. *Int J Cardiol*. 2013;168(2):754–759.
- Bojesen A, Gravholt CH. Morbidity and mortality in Klinefelter syndrome (47,XXY). *Acta Paediatr*. 2011;100(6):807–813.
- Swerdlow AJ, Higgins CD, Schoemaker MJ, Wright AF, Jacobs PA; United Kingdom Clinical Cytogenetics Group. Mortality in patients with Klinefelter syndrome in Britain: a cohort study. *J Clin Endocrinol Metab*. 2005;90(12):6516–6522.
- Gravholt CH, Jensen AS, Høst C, Bojesen A. Body composition, metabolic syndrome and type 2 diabetes in Klinefelter syndrome. *Acta Paediatr*. 2011;100(6):871–877.
- Bojesen A, Høst C, Gravholt CH. Klinefelter's syndrome, type 2 diabetes and the metabolic syndrome: the impact of body composition. *Mol Hum Reprod*. 2010;16(6):396–401.
- Ishikawa T, Yamaguchi K, Kondo Y, Takenaka A, Fujisawa M. Metabolic syndrome in men with Klinefelter's syndrome. *Urology*. 2008;71(6):1109–1113.
- Bojesen A, Kristensen K, Birkebaek NH, Fedder J, Mosekilde L, Bennett P, Laurberg P, Frystyk J, Flyvbjerg A, Christiansen JS,

- Gravholt CH. The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. *Diabetes Care*. 2006;29(7):1591–1598.
20. Bardsley MZ, Falkner B, Kowal K, Ross JL. Insulin resistance and metabolic syndrome in prepubertal boys with Klinefelter syndrome. *Acta Paediatr*. 2011;100(6):866–870.
 21. Aksglaede L, Molgaard C, Skakkebaek NE, Juul A. Normal bone mineral content but unfavourable muscle/fat ratio in Klinefelter syndrome. *Arch Dis Child*. 2008;93(1):30–34.
 22. Ratcliffe SG. The sexual development of boys with the chromosome constitution 47,XXY (Klinefelter's syndrome). *Clin Endocrinol Metab*. 1982;11(3):703–716.
 23. Davis S, Lahlou N, Bardsley M, Temple MC, Kowal K, Pyle L, Zeitler P, Ross J. Gonadal function is associated with cardiometabolic health in pre-pubertal boys with Klinefelter syndrome [published online ahead of print September 16, 2016]. *Andrology*. doi: 10.1111/andr.12275.
 24. Traish AM, Haider A, Doros G, Saad F. Long-term testosterone therapy in hypogonadal men ameliorates elements of the metabolic syndrome: an observational, long-term registry study. *Int J Clin Pract*. 2014;68(3):314–329.
 25. Zitzmann M. Testosterone deficiency, insulin resistance and the metabolic syndrome. *Nat Rev Endocrinol*. 2009;5(12):673–681.
 26. Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N; Testosterone Gel Study Group. Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. *J Clin Endocrinol Metab*. 2000;85(8):2839–2853.
 27. Orr R, Fiatarone Singh M. The anabolic androgenic steroid oxandrolone in the treatment of wasting and catabolic disorders: review of efficacy and safety. *Drugs*. 2004;64(7):725–750.
 28. Hall J, Froster-Iskanian U, Allanson J. *Handbook of Normal Physical Measurements*. Oxford, England: Oxford University Press; 1989.
 29. Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, Bembien DA. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol*. 1988;60(5):709–723.
 30. Laurson KR, Eisenmann JC, Welk GJ. Body fat percentile curves for U.S. children and adolescents. *Am J Prev Med*. 2011;41(4, Suppl 2): S87–S92.
 31. Horan M, Gibney E, Molloy E, McAuliffe F. Methodologies to assess paediatric adiposity. *Ir J Med Sci*. 2015;184(1):53–68.
 32. Freedman DS, Horlick M, Berenson GS. A comparison of the Slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular disease risk factor levels in children. *Am J Clin Nutr*. 2013;98(6):1417–1424.
 33. Greulich W, Pyle SI. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*. 2nd ed. Stanford, CA: Stanford University Press; 1959.
 34. de Ferranti SD, Gauvreau K, Ludwig DS, Neufeld EJ, Newburger JW, Rifai N. Prevalence of the metabolic syndrome in American adolescents: findings from the Third National Health and Nutrition Examination Survey. *Circulation*. 2004;110(16):2494–2497.
 35. Going SB, Lohman TG, Cussler EC, Williams DP, Morrison JA, Horn PS. Percent body fat and chronic disease risk factors in U.S. children and youth. *Am J Prev Med*. 2011;41(4, Suppl 2): S77–S86.
 36. Pięłowska M, Kostka T, Drygas W, Jegier A, Leszczyńska J, Bill-Bielecka M, Kwaśniewska M. Body composition, nutritional status, and endothelial function in physically active men without metabolic syndrome: a 25 year cohort study. *Lipids Health Dis*. 2016;15(1):84.
 37. Corona G, Giagulli VA, Maseroli E, Vignozzi L, Aversa A, Zitzmann M, Saad F, Mannucci E, Maggi M. Testosterone supplementation and body composition: results from a meta-analysis of observational studies. *J Endocrinol Invest*. 2016;39(9):967–981.
 38. Ross JL, Mazzocco MM, Kushner H, Kowal K, Cutler GB, Jr, Roeltgen D. Effects of treatment with oxandrolone for 4 years on the frequency of severe arithmetic learning disability in girls with Turner syndrome. *J Pediatr*. 2009;155(5):714–720.
 39. Wilson DM, McCauley E, Brown DR, Dudley R; Bio-Technology General Corporation Cooperative Study Group. Oxandrolone therapy in constitutionally delayed growth and puberty. *Pediatrics*. 1995;96(6):1095–1100.
 40. Bardsley MZ, Kowal K, Gamber R, Lahlou N, Ross JL. *Androgen Replacement in Boys with 47,XXY Klinefelter syndrome: Influence on the Testicular Phenotype*. Chicago, IL: Endocrine Society; 2014.
 41. Thirumalai A, Rubinow KB, Page ST. An update on testosterone, HDL and cardiovascular risk in men. *Clin Lipidol*. 2015;10(3): 251–258.