Oxandrolone in growth hormone-treated girls with Turner syndrome

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The effect of oxandrolone on voice frequency in growth hormone-treated girls with Turner syndrome

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Abstract

Objectives/hypothesis: Oxandrolone (Ox) increases height gain but may also cause voice deepening in growth hormone (GH)-treated girls with Turner syndrome (TS). We assessed the effect of Ox on objective and subjective speaking voice frequency in GH-treated girls with TS.

Study Design: A multicenter, randomized, placebo-controlled, double-blind study was conducted.

Methods: 133 patients were included and treated with GH (1.33 mg/m^2/day) from baseline, combined with placebo (Pl) or Ox in a low (0.03 mg/kg/day) or conventional (0.06 mg/kg/day) dose from the age of eight, and estrogens from the age of twelve. Yearly from starting Ox/Pl until six months after discontinuing GH+Ox/Pl, voices were recorded and questionnaires were completed.

Results: At start, mean (±SD) voice frequency SDS was high for age (1.0±1.2, $p<0.001$), but normal for height. Compared with GH+Pl, voices tended to lower on GH+Ox 0.03 ($P=0.09$), and significantly lowered on GH+Ox 0.06 ($P=0.007$). At the last measurement, voice frequency SDS was still relatively high in group GH+Pl (0.6±0.7, $p=0.002$), but similar to healthy girls in both GH+Ox groups. Voice frequency became < -2 SDS in one patient (3%) on GH+Ox 0.03, and three patients (11%) on GH+Ox 0.06. The percentage of patients reporting subjective voice deepening was similar between the dosage groups.

Conclusions: Untreated girls with TS have relatively high-pitched voices. The addition of Ox to GH decreases voice frequency in a dose-dependent way. Although most voice frequencies remain within the normal range, they may occasionally become lower than -2 SDS, especially on GH+Ox 0.06 mg/kg/day.

Introduction

Turner syndrome (TS) is a disorder in females that is caused by the complete or partial absence of the second sex chromosome. It is one of the most common chromosomal disorders, affecting approximately one in 2000 live-born girls.[1] The main characteristics are gonadal dysgenesis and short stature. Other possible features are congenital heart disease, renal anomalies and a number of dysmorphic features, including webbed neck, cubitus valgus and lymphedema of the hands and feet.[2] Because the ovaries usually start to involute within 4 or 5 months of gestation,[3] the majority of patients is infertile, has diminished ovarian estrogen and androgen production,[4,5] and needs estrogen replacement therapy to induce pubertal maturation. Untreated adult patients are on average 20 cm shorter than healthy women,[6] mainly due to haploinsufficiency of the Short stature HomeobOX-containing (SHOX) gene.[7] Even though patients are not growth hormone (GH) deficient, GH therapy increases adult height, and the addition of the weak androgen oxandrolone (Ox) to GH may further increase height.[8] Ox is a synthetic, nonaromatizable anabolic androgenic steroid with the chemical name 17β-hydroxy-17α-methyl-2-oxa-5α-androstane-3-one. It is derived from testosterone, in which a carbon atom (at position 2 in the phenanthrene nucleus) is replaced by an oxygen atom. In comparison with testosterone, Ox has a high anabolic to androgenic ratio (10:1).[9] Because Ox dosages ≥ 0.1 mg/kg/day gave rise to in virilizing side effects, including voice deepening,[10] the recommended Ox dosage is nowadays ≤ 0.05 mg/kg/day.[11] It is however unclear whether Ox at dosages < 0.1 mg/kg/day may also result in undesirable voice deepening.

To assess efficacy and safety of Ox in a low (0.03 mg/kg/day) and previously conventional dosage (0.06 mg/kg/day) in GH-treated girls with TS, a randomized, placebo-controlled, double-blind study was performed. In a previous article, we showed that the addition of Ox 0.03 mg/kg/day modestly increases adult height gain and has a fairly good safety profile, whereas Ox 0.06 mg/kg/day does not significantly change adult height gain.[8] In the present article, we describe the effect of Ox on subjective and objective voice frequency by analyzing the voice recordings and questionnaires that were performed and completed yearly during this study.

Materials and Methods

Study setting and participants

Participants were recruited in ten pediatric endocrine centers in the Netherlands from December 1991 to June 2003. Inclusion criteria were: a karyotype associated with TS (except for cytogenetical evidence of Y-chromosomal material); a calendar age between 2.00-15.99 years; and a bone age younger than 12.00 years.[12] Exclusion criteria were: growth failure due to other causes; use of drugs that could interfere with growth; and previous GH, sex hormone, or androgen therapy. The study was performed in accordance with the World Medical Association Declaration of Helsinki and approved by the ethics committee of each participating center. Before enrolment, written informed consent was obtained for each patient.

Treatment

Patients were included in age group 1 (2.00-7.99 years), 2 (8.00-11.99 years), or 3 (12.00-15.99 years). After stratification for calendar age and height SD score (SDS),[13] they were randomized by a computer-generated schedule with a block-size of six, and blindly assigned to receive, orally at bedtime after reaching the age of 8 years, Ox 0.03 mg/kg/day (Ox 0.03) (S.p.A., Milano, Italy), Ox 0.06 mg/kg/day (Ox 0.06), or a similar appearing placebo (Pl). The capsules were manufactured and distributed by one hospital pharmacy. All patients and doctors were blinded for the allocation of the patients, and will remain so until the last patient will finish the study. Only the independent pharmacist (Dr. C.M.A. Rademaker), the statistician (M.A.d.R.), and data analyst (L.A.M., from 2008 onwards) saw unblinded data, but none of them had any contact with the participants. From baseline onwards, biosynthetic human GH (1.33 mg/m^2 body-surface/day, at 1 m^2 equivalent to 46 µg/kg/day) was administered subcutaneously at bedtime. Genotropin^R (Pfizer Inc, New York, USA) was used in age groups 1 and 2, and Humatrope^R (Eli Lilly, Indianapolis, USA) in age group 3. Ox/Pl was started at the age of eight after a number of complete years of GH therapy (i.e. at their main 'year-visit') in age group 1, and at inclusion in age groups 2 and 3 (i.e. between the age of 8.0-16.0 years). In the absence of spontaneous puberty (Tanner breast stage < 2 (B2)),[14] estrogens were started at the age of 12.0-12.99 after

a number of complete years of GH therapy in age groups 1 and 2, and at inclusion in age group 3 (i.e. between the age of 12.0-16.0 years). 17-ß-estradiol was prescribed in age groups 1 and 2, and ethinyl-estradiol in age group 3 (5 and 0.05 µg/kg/day orally, increased to 10 and 0.1 µg/kg/day after two years, respectively). When ethinyl-estradiol became unavailable after March 2002, 17-ß-estradiol was also prescribed in age group 3. Cyclic progesterone was added after at least two years of estrogen therapy. Doses were adjusted every six months, and GH+Ox/Pl were stopped when height velocity was < 1 cm/six months, or when patients decided to stop because they were satisfied with their height.

Assessments

At starting Ox/Pl therapy, after 6 months, and yearly up to 6 months after discontinuing therapy, questionnaires were completed, and voices were recorded in a quiet room by two trained observers who performed all measurements during the total study-period. The voice recordings were performed from December 1993 (when the equipment became available) onwards, and the questionnaires were completed from December 1996 (when the questionnaires were incorporated in the study) onwards.

Two questionnaires were used. The first was completed once and concerned long-term ear, nose & throat history. It included questions regarding major health problems (i.e. hospitalization and surgery, with special attention to prolonged intubation or recent bronchoscopy), past problems of the voice, and logopedic treatment. The second was completed before each voice recording, and included questions regarding recent respiratory tract infections, smoking habits, medication (with special attention to inhalation drugs), and subjective voice alterations in the preceding period.

The voice recording consisted of a count (at a comfortable pitch and loudness) from 10 to 0, which was taped twice by a Casio DA-7 digital audio tape (DAT)-recorder (frequency range: 10-20.000 Hz). The mean fundamental (i.e. speaking) voice frequency was subsequently assessed by one trained and blinded investigator (S.H.L.v.K.), using Multi-Dimensional Voice Program (Kay Elemetrics Corp., Lincoln Park, New Jersey, USA, a computerized voice analysis system). Mean voice frequency

was expressed as SDS for healthy Dutch girls,[15] using the equation: voice frequency SDS = (measured voice frequency – (347.65 - 20.75 x age + 1.439 x age^2 - 0.0408 x age^3)) / (26 - (age - 3) x 0.567). Voice frequencies greater than 2 SDS and smaller than -2 SDS represent unusually high-pitched and low-pitched voices, respectively.

All virilizing adverse events reported by the patient, parent, or medical doctor were registered. Height was measured at every half-yearly visit using a Harpenden stadiometer. The mean of four measurements was used for the analysis, and expressed as SDS for healthy Dutch girls[16]. After discontinuing GH+Ox/Pl therapy, patients were seen at two subsequent year-visits to measure residual growth. Adult height was defined as the last measured height after discontinuing GH.

Statistical analysis

The primary goal of present analysis was to assess the effect of GH+Ox 0.03 and GH+Ox 0.06 vs. GH+Pl on change in objective and subjective voice frequency. Secondary goals were to assess mean voice frequency of untreated girls with TS; the influence of karyotype, spontaneous puberty, and height on voice frequency change during Ox/Pl therapy; and the correlation between objective and subjective voice change, as well as between objective voice change and virilizing adverse events other than voice lowering.

A modified intention-to-treat analysis was performed in which patients who refused starting Ox/Pl were excluded. When Ox/Pl was discontinued before GH, the moment GH was discontinued was identified as 'at discontinuing GH+Ox/Pl'. Values for untreated girls with TS were obtained using the baseline values of girls from age groups 2 and 3 because these groups, in contrast to age group 1, were not treated with GH before starting Ox/Pl. To compare untreated voice frequency SDS with that of (younger) girls with the same height, 'age' was replaced by 'height age' (i.e. the average age of healthy Dutch girls[16] corresponding to the height of the patient) in the equation for calculating voice frequency SDS.

Because voice frequency has been suggested to be associated with the degree of *SHOX* haploinsufficiency,[17] the karyotypes of the patients were categorized into two groups based on the total absence (45,X; 46,X,Xi(Xq); 45,X/46,X,Xi(Xq)) or partial presence (all other karyotypes) of the p-arm of the second X chromosome.

Subjective voice deepening was defined as any of the following responses: the voice had become deeper, lower or less high-pitched, had gotten a more grown-up character, and/or the patient had trouble reaching high notes. For the analysis on subjective voice alterations, also the questionnaires belonging to voice recordings that could not be analyzed due to technical problems were used. Virilizing adverse events (other than voice deepening) included clitoral enlargement and an increase in body hair, both previously identified as Ox-related virilization[10,18-20]). These events were included in the analysis if a girl reported these complaints at least twice (i.e. either the same complaint at two visits, or two complaints at one visit), or if the girl decided to discontinue Ox/Pl because of the event.

Differences between dosage groups were tested by linear regression using two dummies (for GH+Ox 0.03 and GH+Ox 0.06), and differences in proportions by Pearson χ^2 tests and Fisher's exact tests. Means were compared with zero by a one-sample t test. Differences in change of outcome variables during the study period were assessed by repeated measurements analysis. Models were fitted with a different intercept and slope per dosage group and a random intercept and slope per patient. Results are presented as means±SD and differences assessed by repeated measurements analyses as means, SE. A p value less than 0.05 was considered significant.

Results

Characteristics of the patients

Fig. 1 shows the 133 patients that were randomized. Twenty-one girls were excluded from the analysis: four were non-compliant and lost to follow-up, nine were still treated, and eight refused to start Ox/Pl therapy. The voices of 24 girls were either not recorded or not analyzed due to logistic and/or technical problems. Of the remaining 88 patients, a total of 418 voice recordings were available for the analysis. Table I shows the characteristics of the patients at starting GH and Ox/Pl, which were similar between the three dosage groups.

The questionnaire concerning ear, nose & throat history was filled out by 88% (77/88) of the patients. It revealed that 42% (32/77) of the patients had received logopedic therapy prior to their first voice recording and that these patients were evenly distributed over the three dosage groups. Logopedic therapy was started at a mean age of 6.0±1.6 years and was continued for a mean of 1.6±1.1 years, with five patients still following therapy when Ox/Pl was started. The yearly questionnaire regarding recent ear, nose & throat history was filled out before 75% (314/418) of the voice recordings. It revealed that none of the patients had voice complaints due to a concurrent respiratory tract infection. Two patients, one on GH+Ox 0.03 and one on GH+Ox 0.06, confirmed smoking sigarettes (fifteen and four sigarettes per day, respectively).

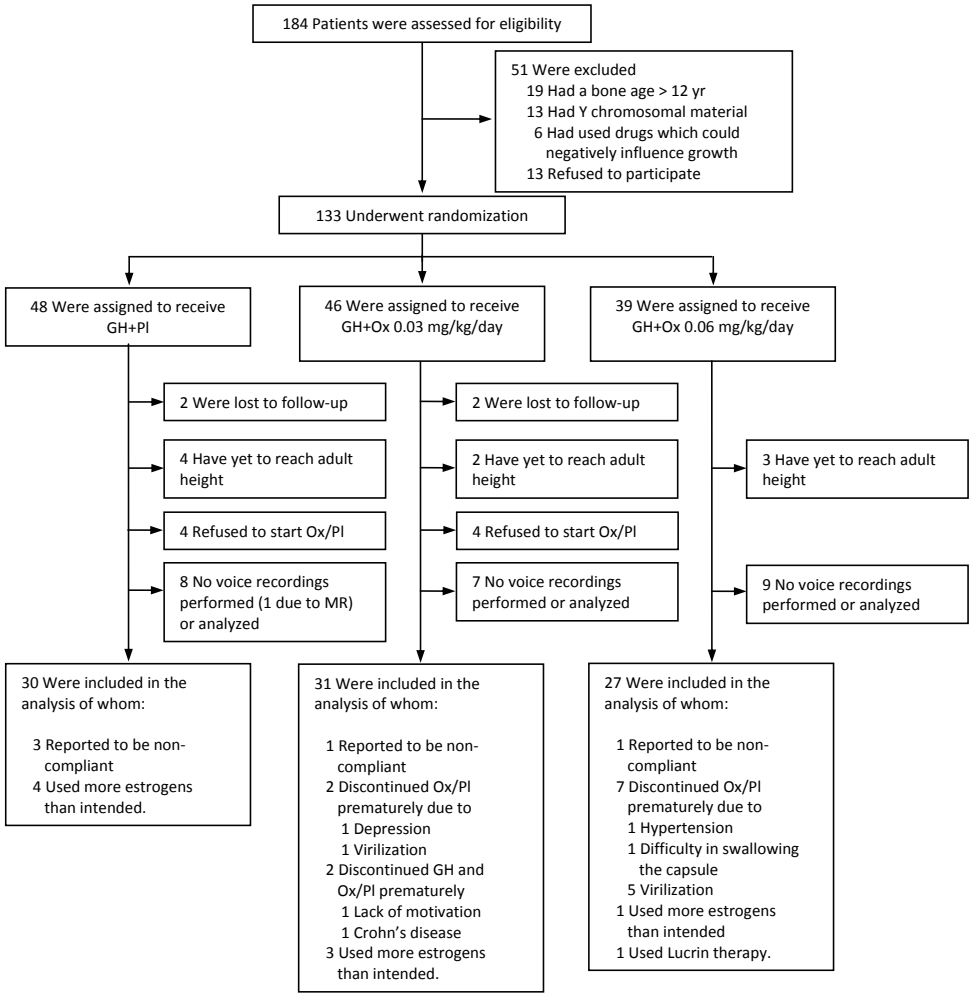

Figure 1. Enrollment, randomization and analysis of the patients.

GH, growth hormone; Pl, placebo; Ox 0.03, oxandrolone 0.03 mg/kg/day; Ox 0.06, oxandrolone 0.06 mg/kg/day; MR, mental retardation.

Table I. Characteristics and treatment per dosage group.*

Characteristic	GH+Pl (N =30)	GH+Ox 0.03 (N =31)	GH+Ox 0.06 (N =27)
Age at starting GH – yr	9.2±3.7	8.3±3.6	8.0±3.2
Height at starting GH – SDS§	-3.0±0.7	-3.0±0.7	-2.8±0.7
Age at starting Ox/Pl – yr	10.4±2.2	9.7±2.0	9.5±1.7
Age at starting estrogens or at Tanner stage B2 – yr†	12.3±1.3	12.3±0.8	12.0±0.8
Karyotype, 45,X; 46,X,Xi(Xq); ad 45,X/46,X,Xi(Xq) – n (%)	19 (63%)	19 (61%)	18 (67%)
Karyotype, other – n (%)	11 (37%)	12 (39%)	9 (33%)
Puberty developed spontaneously – n (%)	9 (30%)	7 (23%)	8 (30%)
Puberty was induced – n (%)	21 (70%)	24 (77%)	19 (70%)
Duration of GH therapy – yr	6.4±3.0	6.7±2.8	6.5±2.8
Duration of Ox/Pl therapy – yr	5.2±1.5	5.0±1.6	4.4±1.7
Age at discontinuation of GH+Ox/Pl – yr	15.7±1.2	15.0±1.3	14.5±1.1
Age at last visit – yr	17.7±1.4	16.8±1.3	16.8±1.1
Adult height – SDS‡	-2.3±0.8	-2.1±1.2	-2.2±0.9

* Values are expressed as means±SD, unless otherwise indicated.
§ Height SDS was calculated using Dutch references.
† If puberty was induced, the moment at starting estrogens was used; if puberty developed spontaeously, the moment at Tanner breast stage 2 was used.
‡ Based on reference values of healthy Dutch girls. Reference data for the age of 21 rather than the actual age were used to avoid overestimation in patients that stopped growing at an earlier age than the reference group.

Objective voice frequency

Fig. 2 shows the voice frequency SDS of the individual patients before, during, and after discontinuing Ox/Pl therapy. Mean voice frequency SDS of the untreated patients at baseline (from age groups 2 and 3) was higher than that of healthy girls of the same age (mean±SD: 1.1±1.2 SDS, P<0.001), but close to the mean for girls of the same height (0.3±1.1 SDS, P=0.1).

During Ox/Pl therapy, voice frequency SDS lowered significantly (mean, SE: -0.16, 0.03 SDS/yr, P<0.0001). Voice deepening tended to be greater on GH+Ox 0.03 (P=0.09), and was significantly greater on GH+Ox 0.06 (P=0.007) than on GH+Pl. During the study, five voice recordings of two girls displayed voice frequencies higher than +4.5 SDS (Fig. 2). No special explanation was found for these exceptionally high voices, except that both girls had a moderate to severe hearing impairment and followed special education. When excluding these voice recordings as outliers, voice deepening was similar on GH+Ox 0.03 as on GH+Pl (P=0.2), but still significantly greater on GH+Ox 0.06 than on GH+Pl (P=0.02) (Table II). Four patients, one (3%) on GH+Ox 0.03, and three (11%) on GH+Ox 0.06 had a voice frequency less than -2 SDS at least once during the study. The girl on GH+Ox 0.03 (who smoked 15 cigarettes per day) had a voice frequency of -2.3 SDS after discontinuing GH+Ox; two girls on GH+Ox 0.06 had a voice frequency of -2.3 and -2.7 SDS, respectively, after discontinuing GH+Ox; and the third girl on GH+Ox 0.06 had voice frequencies of -2.6 and -4.1 SDS during GH+Ox, and -4.2 SDS after discontinuing GH+Ox (Fig. 2). Whereas in group GH+Pl, mean voice frequency SDS at the last measurement (i.e. at or after discontinuation of Ox/Pl therapy) was still higher than in the reference population (+0.6±0.7, P=0.002), it was comparable with the reference population in groups GH+Ox 0.03 (0.3±1.2, P=0.3) and GH+Ox 0.06 (-0.2±1.6, P=0.6) (Table II).

Factors influencing objective voice frequency other than oxandrolone therapy

When looking at the untreated patients at baseline, the voices of patients with monosomy and isochromosome karyotypes were significantly higher than those of patients with other karyotypes (P=0.03). The presence of spontaneous puberty (correlated with karyotype, P<0.001) also influenced voice frequency. The voices of girls in whom puberty was induced tended to be higher at starting Ox/Pl than those of girls in whom puberty developed spontaneously (P=0.06). Corrected for

Ox/Pl dosage-group, none of the following variables significantly influenced voice deepening during Ox/Pl therapy: age at starting Ox/Pl, spontaneous puberty, karyotype, and height.

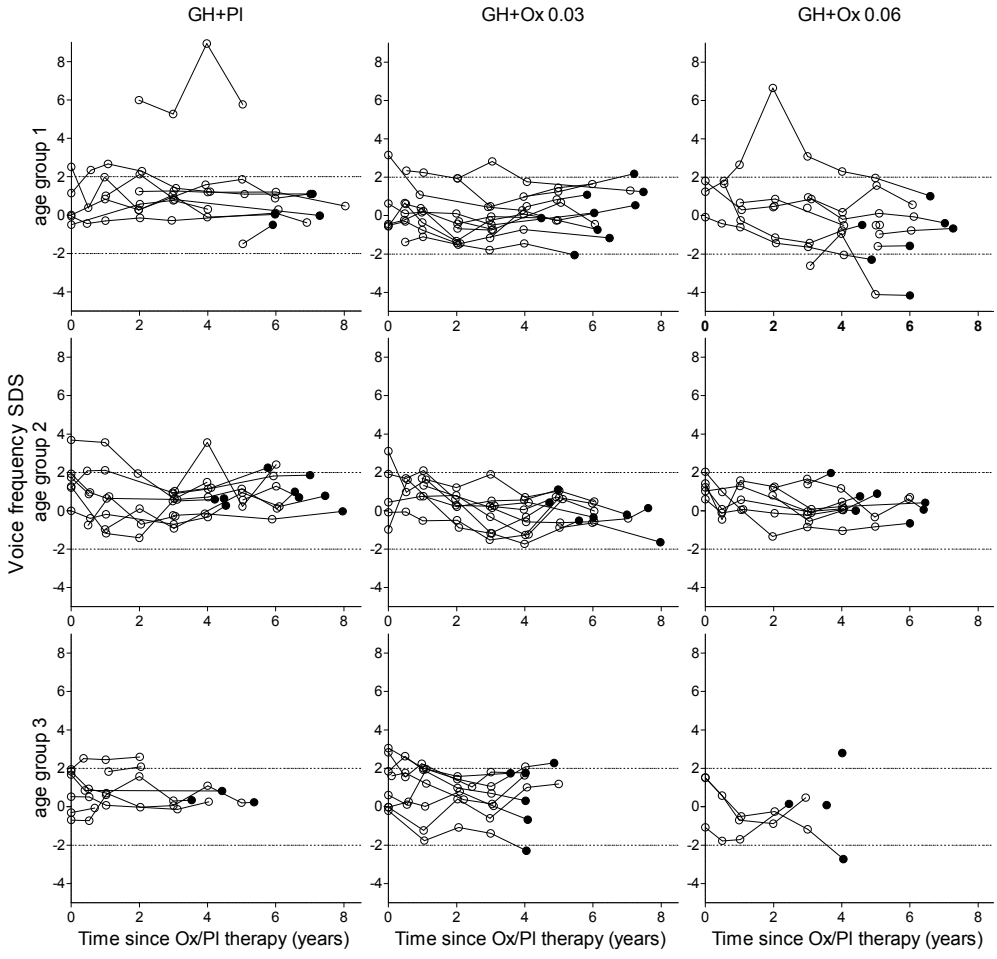

Figure 2. Voice frequency SDS of the individual patients since starting Ox/Pl therapy per age group and dosage group.

All available voice recordings are shown. Open circles represent voice frequencies during Ox/Pl, filled circles represent voice frequencies after discontinuation of Ox/Pl therapy. Note that GH had been started prior to the start of Ox/Pl therapy in age-group 1. The recordings were included in repeated measurements analyses, which takes into account missing data.

Subjective voice frequency

A total of 420 questionnaires regarding short-term ear, nose & throat history were filled out by 88 patients (100%). These questionnaires consisted of 314 questionnaires that were completed before a voice recordings that was used in the analyses, and 106 questionnaires belonging to voice recordings that could not be analyzed due to technical problems. Subjective voice deepening during Ox/Pl was reported in 33% of patients on GH+Pl, 42% on GH+Ox 0.03, and 52% on GH+Ox 0.06, frequencies which were not significantly different between the dosage groups (Table II, Fig. 3). A further six girls (one from GH+Pl, three from GH+Ox 0.03 and two from GH+Ox 0.06) reported that their voice had always been low and did not change during therapy. Voice alterations other than subjective voice deepening were infrequently reported (Fig. 3).

The decrease in objective voice frequency was greater in patients who reported subjective voice deepening than in those who did not (mean, SE: -0.24, 0.05 SDS/yr vs. -0.10, 0.04 SDS/yr, P=0.02), whereas girls who reported hirsutism and/or mild clitoral enlargement (Table II) had a similar decrease in objective voice frequency than those who did not (mean, SE: -0.16, 0.08 SDS/yr vs. -0.15, 0.04 SDS/yr). Of the four patients with a voice frequency below -2 SDS (Fig. 2), only two reported subjective voice deepening: one girl on GH+Ox 0.03, and one on GH+Ox 0.06. The latter girl discontinued Ox 0.06 prematurely due to hirsutism and clitoromegaly. After discontinuing Ox, she followed logopedic therapy because she had a monotonous voice and slow oral motor performance. She reported being frequently bullied because of her low voice. Another girl (from group Ox 0.06) reported that her voice was sometimes low after discontinuing GH+Ox, but that she had not noticed any voice lowering during therapy.

Table II. Objective and subjective voice frequency and virilizing adverse events per dosage group.

Outcome	GH+Pl (N = 30)	GH+Ox 0.03 (N = 31)	GH+Ox 0.03 vs. GH+Pl P Value	GH+Ox 0.06 (N = 27)	GH+Ox 0.06 vs. GH+Pl P Value
Voice frequency at starting Ox/Pl (all age groups) – SDS†‡	1.1±1.2**	0.9±1.4**		1.0±0.9**	
Age group 1 (after previous GH therapy) – SDS†	0.6±1.2	0.4±1.6		1.0±1.0	
Age groups 2 and 3 (at baseline) – SDS†	1.2±1.2**	1.1±1.4**		1.0±0.9**	
Change in voice frequency during Ox/Pl (all age groups) – SDS/yr, SE§	-0.05, 0.05	-0.17, 0.05	0.09	-0.27, 0.06	0.007
Age group 1 (after previous GH therapy) – SDS/yr, SE	-0.02, 0.08	-0.13, 0.09	0.3	-0.29, 0.10	0.04
Age groups 2 and 3 – SDS/yr, SE	-0.06, 0.07	-0.21, 0.06	0.1	-0.22, 0.09	0.2
Change in voice frequency during Ox/Pl without outliers – SDS/yr, SE¶	-0.07, 0.05	-0.16, 0.05	0.2	-0.26, 0.06	0.02
Voice frequency at the last measurement – SDS	0.6±0.7**	0.3±1.2	0.4	-0.2±1.6	0.06
Girls reporting subjective voice deepening – n (%)	10 (33%)	13 (42%)	0.5	14 (52%)	0.2
Girls reporting virilization other than voice deepening during Ox/Pl – n (%)‖	1 (3%)	5 (16%)	0.2	8 (30%)	0.01
Hirsutism	1 (3%)	4 (13%)	0.4	8 (30%)	0.01
Mild clitoromegaly	0 (0%)	4 (13%)	0.1	4 (15%)	0.04

* values are expressed as means±SD, unless otherwise indicated.

† No statistical tests were applied to assess differences between the dosage groups at starting Ox/Pl.

‡ Based on 18, 19 and 11 tests in GH+Pl, GH+Ox 0.03 and GH+Ox 0.06, respectively. These recordings were included in repeated measurements analyses, which takes into account missing data.

§ Based on 123, 149 and 92 tests during Ox/Pl and 20, 26, 21 tests at the last measurement (i.e. at or after discontinuation of Ox/Pl) in GH+Pl, GH+Ox 0.03 and GH+Ox 0.06, respectively.

¶ Five voice recordings (of two girls) that displayed a voice frequency higher than 4.5 SDS were identiefied as outliers.

‖ Some patients reported hirsutism as well as clitoromegaly, while others reported only one these complaints. The number of the patients reporting both adverse event may therefore not equal the numbers in this row.

** Significantly different from 0 SDS (i.e. from healthy Dutch girls, P<0.05).

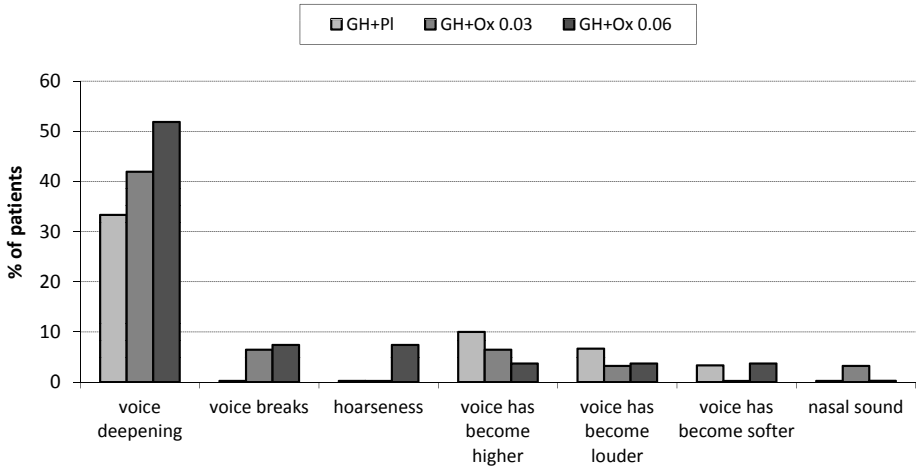

Figure 3. Subjective voice alterations reported during therapy.

Discussion

This randomized, placebo-controlled, double-blind study shows that Ox 0.03 and 0.06 increase voice deepening in GH-treated girls with TS in a dose-dependent way. It furthermore shows that untreated girls with TS, especially those with monosomy and isochromosome karyotypes, have higher-pitched voices than healthy girls. The voices remained relatively high-pitched on GH+Pl therapy, but became comparable with those of healthy girls on GH+Ox 0.03 or 0.06, except for the voices of one patient (3%) on GH+Ox 0.03 and three patients (11%) on GH+Ox 0.06, which became lower than -2 SDS. The percentage of patients reporting subjective voice deepening was similar between the dosage groups. Objective voice deepening was greater in patients that reported subjective voice deepening, but similar between girls who did and did not show other signs of virilization.

Consistent with earlier reports,[17,21,22] we showed that untreated patients with TS have significantly higher voice frequencies than healthy girls of the same age. We think that three mechanisms may underlie this finding. First, because *SHOX* is expressed in the first and second pharyngeal arches,[23] *SHOX*-haploinsufficiency in TS may disturb normal growth in the craniofacial regions, causing the larynx

to be smaller,[17] and voice frequency to be higher.[23] This mechanism would also clarify why monosomic and isochromosomic patients (who are completely *SHOX*-haploinsufficient) have higher voices than patients with other karyotypes.[17] Second, we found that voice frequencies of untreated patients were similar to those of younger girls with similar heights, suggesting that the short stature of the patients may play a role. Although our finding could also indicate that girls with TS are physically 'younger' than healthy girls in some other way than their height, height is thought to be one of the predictive factors for the voice pitch of children.[15,24] Finally, the partial androgen insufficiency in TS may play a role. Androgen levels are up to 60% lower in 15 year old TS patients, especially in girls with TS in whom puberty needs induction.[4]

We found that Ox 0.03 tended to increase voice deepening and that Ox 0.06 significantly increased voice deepening. Earlier reports, consisting of smaller numbers of patients[21,22] or cross-sectional data,[17] also suggested that androgen and/or GH therapy decreases voice frequency.[17,21,22] Another study showed that voice deepening was found in 7 of the 44 patients on GH+Ox 0.1 mg/kg/day.[10] The most likely explanation of the voice deepening capacity of Ox is that it directly virilizes the vocal cords, analogous to androgen therapy in adult women in whom an increase in muscle mass, extensibility, and length of the vocal folds was found.[25-28] This hypothesis is further supported by the recent detection of androgen receptors in the vocal folds.[29] Although one may hypothesize that also the growth-promoting effect of Ox[23,30] may augment the voice-deepening capacity of Ox, we found no significant influence of height on voice deepening during Ox/Pl therapy.

Although our data confirm some earlier suggestions that voice frequency 'normalizes' during Ox and/or GH therapy.[17,21] this was not the case for one patient on GH+Ox 0.03 and three patients on GH+Ox 0.06 who developed voice frequencies below -2 SDS. Except for the initial changes,[27] androgen-induced voice lowering is thought to be irreversible,[17,22] and only voice exercises may be of some benefit.[28] The finding that the voices of 11% of the patients on GH+Ox 0.06 became lower than -2 SDS confirms our previous finding that Ox 0.06 can result in virilization in a considerable proportion of patients.[8] In this report, we discouraged the use of Ox 0.06 in GH-treated girls with TS because of the suboptimal efficacy-safety ratio of

Ox 0.06. We however think that the voice frequency of -2.3 SDS measured once in a girl on GH+Ox 0.03 should not be considered a strong argument against the use of Ox 0.03. The smoking habits of the girl may have had an additional voice-lowering effect (by tobacco induced Reinke's edema of the vocal folds[31]), and the frequency of one out of 31 patients (3%) was close to the statistically expected frequency of 2.3%. Although to our knowledge, no data are available on the relation between quality of life and voice-pitch, we hypothesize that a significantly high voice frequency may be as undesirable as a significantly low voice frequency. This may especially be true for patients with TS, who are often perceived as younger than their actual age.[32] In this respect, the voice lowering in patients treated with GH+Ox 0.03, which results in a mean adult voice frequency close to the mean of the normal population, may be regarded favorable for some girls.

The questionnaires concerning subjective voice changes showed that the percentage of patients reporting subjective voice deepening was similar between the dosage groups. Our data however show that a questionnaire concerning subjective voice changes is not a reliable measure for undesirable voice deepening because it has a low sensitivity and specificity. The low sensitivity was shown by the fact that only two of the four girls with a voice frequency lower than -2 SDS reported voice deepening during Ox/Pl. The low specificity is reflected by the finding that subjective voice deepening also covered physiological voice deepening during childhood, illustrated by the fact that also 33% of girls on GH+Pl reported voice deepening. Whereas objective voice deepening was greater in patients reporting voice deepening, patients with hirsutism and/or mild clitoromegaly did not show greater voice deepening than girls without these complaints. We therefore could not confirm the previous suggestion that voice deepening may precede other androgenic effects.[33]

To the best of our knowledge, this study is the first randomized, placebo-controlled, double blind study to assess the effect of Ox 0.03 and 0.06 on voice frequency in GH-treated girls with TS. Unfortunately, not all voice recordings were performed or analyzed due to logistic and technical problems. In our view, our findings are however still valid because voice recordings were missing completely at random and because all available voice recordings were analyzed using repeated measurements

(a method that takes into account missing data). Another shortcoming is that the questionnaire regarding subjective voice alterations was incorporated in the study while a part of the patients was already enrolled in the trial. The actual correlation between subjective and objective voice deepening may therefore be somewhat stronger than we found.

Conclusion

We conclude that the voices of untreated girls with TS are relatively high-pitched and that the addition of Ox to GH increases voice deepening in a dose-dependent way. Although most voice frequencies have remained within the normal range, they may occasionally become lower than -2 SDS, especially on GH+Ox 0.06 mg/kg/day. Our data confirm previous findings that Ox 0.03 has an acceptable safety profile, whereas Ox 0.06 results in virilization in a considerable number of patients. Although objective voice deepening was greater in patients reporting subjective voice deepening, a questionnaire concerning subjective voice changes does not seem a reliable measure for undesirable voice deepening.

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Declaration of interest

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