Oxandrolone in growth hormone-treated girls with Turner syndrome

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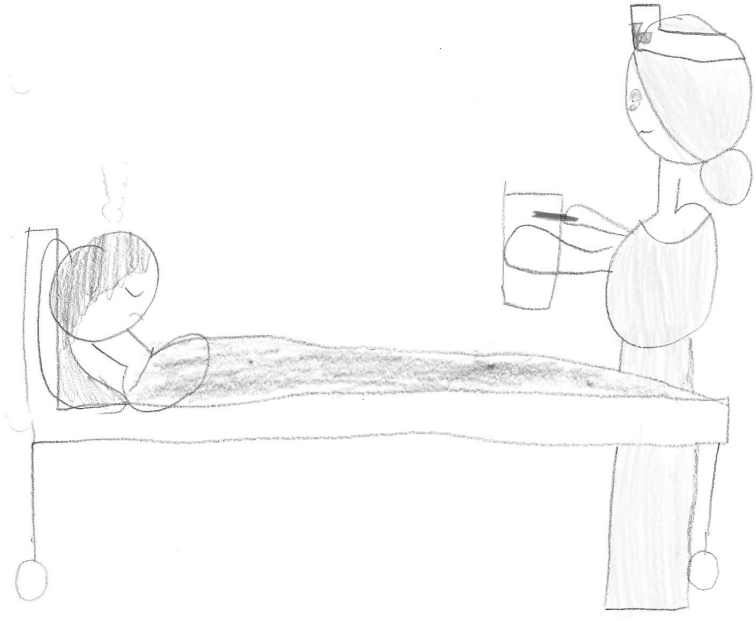
The effect of oxandrolone on glucose metabolism in growth hormone-treated girls with Turner syndrome



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Abstract

Background: The weak androgen oxandrolone (Ox) may increase height but may also affect glucose metabolism in girls with Turner syndrome (TS).

Methods: In a randomized, placebo-controlled, double-blind study we assessed the effect of Ox at a dosage of either 0.06 or 0.03 mg/kg/day on glucose metabolism in 133 GH-treated girls with TS. Patients were treated with GH (1.33 mg/m^2/day) from baseline, combined with placebo (Pl) or Ox from the age of eight, and estrogens from the age of twelve. Oral glucose tolerance tests (OGTT) were performed, and HbA1c levels were measured before, during, and after discontinuing Ox/Pl therapy.

Results: Insulin sensitivity, assessed by the whole-body insulin sensitivity index (WBISI) decreased during GH+Ox/Pl (P=0.003) without significant differences between the dosage groups. Values returned to pre-treatment levels after discontinuing GH+Ox/Pl. On GH+Ox, fasting glucose was less frequently impaired (Ox 0.03, P=0.001; Ox 0.06, P=0.02) and HbA1c levels decreased more (P=0.03 and P=0.001, respectively) than on GH+Pl.

Conclusions: We conclude that in GH-treated girls with TS, Ox at a dosage of 0.03 or 0.06 mg/kg/day does not significantly affect insulin sensitivity. Insulin sensitivity decreases during GH therapy, to return to a pre-treatment level after discontinuing therapy.

Introduction

Women with Turner syndrome (TS) are at an increased risk of impaired glucose tolerance and diabetes mellitus type 2 [1]. Studies in adult patients have pointed to progressive β-cell failure as the primary defect in glucose homeostasis [2, 3], whereas in children, the presence of insulin resistance has been reported by some [4, 5], though not all [6, 7] studies. Furthermore, growth hormone (GH) treatment may further decrease insulin sensitivity, an effect that appears reversible after discontinuing therapy [7-9]. Other studies have suggested that especially the combination of GH and the growth promoting androgen oxandrolone (Ox) may negatively affect glucose metabolism [10-13]. These studies however used Ox at dosages of ≥ 0.06 mg/kg/day, leaving it uncertain whether Ox at a nowadays recommended dosage of ≤ 0.05 mg/kg/day [14] would have the same consequence.

To assess the effect of Ox at a low (0.03 mg/kg/day) and previously conventional (0.06 mg/kg/day) dosage in GH-treated girls with TS, we conducted a randomized, placebo-controlled, double-blind study. 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 does not significantly change adult height gain [15]. We demonstrated that glycosylated hemoglobin (HbA1c) levels remained normal in all girls except one, and that none of the girls developed diabetes mellitus type 1 or 2. In addition, we showed that the addition of Ox to GH may further reduce subcutaneous fat mass, and increase muscle mass, resulting in a fat mass that is lower and a muscle mass that is higher than in healthy girls [16]. In the present article, we focus on the effect of GH+Ox on glucose metabolism in detail by analyzing the yearly performed oral glucose tolerance tests (OGTTs) and yearly measured HbA1c levels.

Materials and Methods

Participants

Participants were recruited in ten pediatric endocrine centers in the Netherlands from December 1991 to June 2003 according to inclusion and exclusion criteria described elsewhere [15]. 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

The treatment regimen has been described previously [15]. In short, 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) [17], they were randomized 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). 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. 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) [18]), 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). 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. Thereafter, patients were followed for two subsequent year-visits to measure growth after discontinuing GH+Ox/Pl.

Assessments

Two trained observers performed all measurements during the total study period. Three-hour oral glucose tolerance tests (OGTT) were performed at starting Ox/Pl, 12 and 24 months thereafter, every other year, or yearly in case of impaired glucose tolerance (IGT), and 6 months after discontinuing GH+Ox/Pl. After an overnight fast and three days of normal physical activity and unrestricted diet containing 50% of the calories in carbohydrate form, the patients were given an oral glucose load of 1.75

g/kg (max 50 g). The evening before the OGTT, no GH was administered. According to The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, impaired fasting glucose (IFG) was defined as a fasting glucose ≥ 5.6 mmol/l (100 mg/dl) and IGT as a 2-h glucose level ≥ 7.8 mmol/l (140 mg/dl) [19]. To assess insulin sensitivity, the Whole-Body Insulin Sensitivity Index (WBISI, also known as ISI_{comp}) was calculated as follows: WBISI = 10,000/√((fasting plasma glucose x fasting plasma insulin) x (mean glucose concentration x mean insulin concentration during 2-hour OGTT)) [20]. This index includes both hepatic and peripheral insulin sensitivity and has been validated in subjects with normal, impaired, and diabetic glucose tolerance using the hyperinsulinemic-euglycemic clamp as the gold standard. It has been found superior to other, more crude measures of insulin sensitivity [20]. In addition, homeostasis model assessment insulin resistance (HOMA-IR) was assessed using the HOMA2 Calculator v2.2 (http://www.dtu.ox.ac.uk/homa) [21]. HOMA-IR is based on simultaneously sampled fasting levels of plasma glucose and insulin, and is thought to primarily indicate hepatic insulin sensitivity [20]. Furthermore, the incremental (i.e. increase above baseline concentration) area under the curve for plasma glucose ($IAUC_{gluc}$) and insulin ($IAUC_{insul}$) levels was calculated using the trapezoid rule. Glycosylated hemoglobin (HbA1c) levels were determined yearly from baseline until 6 months after discontinuing GH+Ox/Pl. Height was measured at every visit using a Harpenden stadiometer. The mean of four measurements was expressed as SDS for healthy Dutch girls [22]. To avoid overestimation of adult height SDS in patients who stopped growing at an earlier age than healthy peers, reference data for the age of 21 instead of the actual age were used for calculating adult height SDS.

Assays

The plasma glucose level was measured at the local hospital laboratories. Plasma insulin was determined in one laboratory by a Radioimmunoassay (Medgenix, Fleurus, Belgium from 1991-2002; Diagnostic Systems Laboratories Inc, Texas, USA from 2003 onwards). Both methods produced identical results. According to this assay insulin concentrations can be converted to pmol/l by multiplying them by 6.89. The upper normal fasting level was < 20 mU/l, the intra-assay coefficient of variation (CV) was 6–10%, and the inter-assay CV was 6–11%. Glycosylated hemoglobin (HbA_{1c})

levels were measured in one laboratory using a dedicated automatic high pressure liquid chromatography analyzer (DIAMAT from 1991-1997, and VARIANT from 1997 onwards; Bio-Rad Laboratories, Inc., Edgemont, CA). Both methods produced identical results. The upper normal assay limit was 6.6% and the combined intra- and inter-assay CV over a three month period was 2.0% at a level of 5%, and 2.5% at a level of 10%.

Statistical analysis

The primary goal was to assess the effect of GH+Ox 0.03 and 0.06 mg/kg/day vs. GH+Pl on the occurrence of IFG and IGT, fasting insulin and glucose levels, insulin sensitivity (measured by WBISI, and in addition by HOMA and $IAUC_{gluc}$ and $IAUC_{insul}$), and HbA1c. A secondary goal was to assess the reversibility of the possible effects.

We performed a modified intention-to-treat analysis in which patients who refused 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. Because WBISI could not be calculated if one of the insulin or glucose values was missing due to hemolysis or logistic problems, up to a maximum of two missing insulin values and two missing glucose values per OGTT were accounted for by single imputation based on the expectation maximization algorithms. Means were compared with zero by a one-sample t test. Differences between dosage-groups were tested by linear regression using two dummies (for groups GH+Ox 0.03 and GH+Ox 0.06), and differences in proportions by Pearson χ^2 tests and Fisher's exact tests. Insulin, HOMA-IR, WBISI, $IAUC_{gluc}$, and $IAUC_{insul}$ were logarithmically transformed to allow parametric testing. Differences in change of outcome variables (during the first two years of Ox/Pl, as well as during the total study period), were assessed by repeated measurements analyses. Linear mixed models were fitted with 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. Unevenly distributed data are presented as a median with a range. A P-value less than 0.05 was considered significant.

Results

Characteristics of the patients

Of the 133 patients that were included in the study, four were lost to follow-up, nine were still treated when the analysis started, and the parents of eight girls refused Ox/Pl because of fear of side effects and/or satisfaction with growth. Leaving out these patients, 112 patients were left for the modified intention-to-treat analysis (a flow chart was published previously [16]). BMI SDS, waist circumference SDS, and the sum of four skinfold-thickness SDS were comparable at starting Ox/Pl [16]. Table 1 shows baseline and treatment characteristics per dosage group. Table 2 shows the outcome variables. A total of 574 OGTTs were performed. Fig. 1 shows the mean glucose and insulin levels during the OGTT per dosage group.

Impaired fasting glucose and impaired glucose tolerance

Although none of the girls developed diabetes mellitus type 1 or 2, 21 of the 112 girls (19%) had IFG, 21 (19%) had IGT, and 6 (5%) had both IFG and IGT at least once during the total duration of the study. In case of IFG, the median (range) of fasting glucose levels was 6.0 mmol/l (5.6-8.9); in case of IGT, the median (range) of the 2-hour glucose levels was 8.6 mmol/l (7.8-12.2). Seven girls already showed IGT and three girls already showed IFG before starting GH+Ox/Pl therapy. The percentage of patients that had IFG at least once during GH+Ox/Pl was smaller on GH+Ox than on GH+Pl (GH+Ox 0.03, P=0.001; GH+Ox 0.06, P=0.02), whereas the percentage of patients that had IGT at least once was not significantly different between the dosage groups (Table 2). After discontinuing GH+Ox/Pl, two patients from group GH+Pl had IFG, and one patient from group GH+Ox 0.03 had IGT. One girl from age group 3, who also had IGT before, and one year after starting GH+Ox 0.03, showed a 2-hour glucose level of 12.2 mmol/l and an elevated HbA1c of 7.7% after two years of therapy. She therefore discontinued GH+Ox 0.03, after which both measures returned to normal values.

Table 1. Characteristics and treatment per dosage group.*

Characteristic	GH+Pl (n =38)	GH+Ox 0.03 (n =38)	GH+Ox 0.06 (n =36)
Age at starting GH – yr	9.7±3.8	9.0±3.8	9.1±3.5
Height at starting GH – SDS†	-2.9±0.7	-2.9±0.6	-2.9±0.7
Age at starting Ox/Pl – yr‡	10.9±2.3	10.2±2.5	10.2±2.2
Karyotype: 45,X – no. (%)	21 (55)	14 (37)	16 (44)
Other – no. (%)‡	17 (45)	24 (63)	20 (56)
Puberty developed spontaneously – no. (%)	9 (24)	8 (21)	9 (25)
Duration of GH therapy – yr	6.1±3.1	6.2±2.9	5.8±2.8
Duration of Ox/Pl therapy – yr§	5.0±1.5	4.8±1.6	4.2±1.7
Age at discontinuation of GH+Ox/Pl – yr	15.8±1.2	15.2±1.5	14.9±1.3
Age at last visit – yr	17.8±1.4	17.0±1.5	17.0±1.2

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

† Height SDS was calculated using Dutch references.

‡ These consisted of the following karyotypes: in group GH+Pl: mosaic (45,X/46,XX, n=5); isochromosome (45,X/46,X,i(X), n=3; 46,X,i(Xq), n=1); deletions (46,X,del(X), n=2); trisomy X (45,X/47,XXX, n=2); ring chromosome (45,X/46,X,r(X), n=3); and marker chromosome (45,X/46,X+mar, n=1). In group GH+Ox 0.03: mosaic (45,X/46,XX, n=2); isochromosome (45,X/46,X,i(Xq), n=5; 46,X,i(Xq), n=6; 45,X/46,XX/46,X,i(Xq), n=1; 46,X,Xp-/46,X,i(Xq), n=1); deletions (45,X/46,X,del(X), n=1; 46,XXq-(q13-qter), n=1); trisomy X (45,X/47,XXX, n=1; 45,X/46,XX/47,XXX, n=2; 45,X/46,X,i(Xq)/47,XXX, n=1); ring chromosome (45,X/46,X,r(X), n=2); and marker chromosome (45,X/46,X+mar, n=1). In group GH+Ox 0.06: mosaic (45,X/46,XX, n=2); isochromosome (45,X/46,X,i(Xq), n=5; 46,X,i(Xq), n=2); deletions (46,X,del(X), n=1; 45,X/46,X,del(X), n=2); ring chromosome (45,X/46,X,r(X), n=4; 45,X/46,XX/46,X,r(X), n=1); marker chromosome (45,X/46,X+mar, n=2); and a translocation karyotype (46,X,+der,t(X;13)(q13;q12.3), n=1).

§ Ox/Pl therapy was started after a full number of years of GH therapy between the age of 8.0 to 8.99 in age group 1, and at inclusion (i.e. between the age of 8 and 16 years) in age groups 2 and 3.

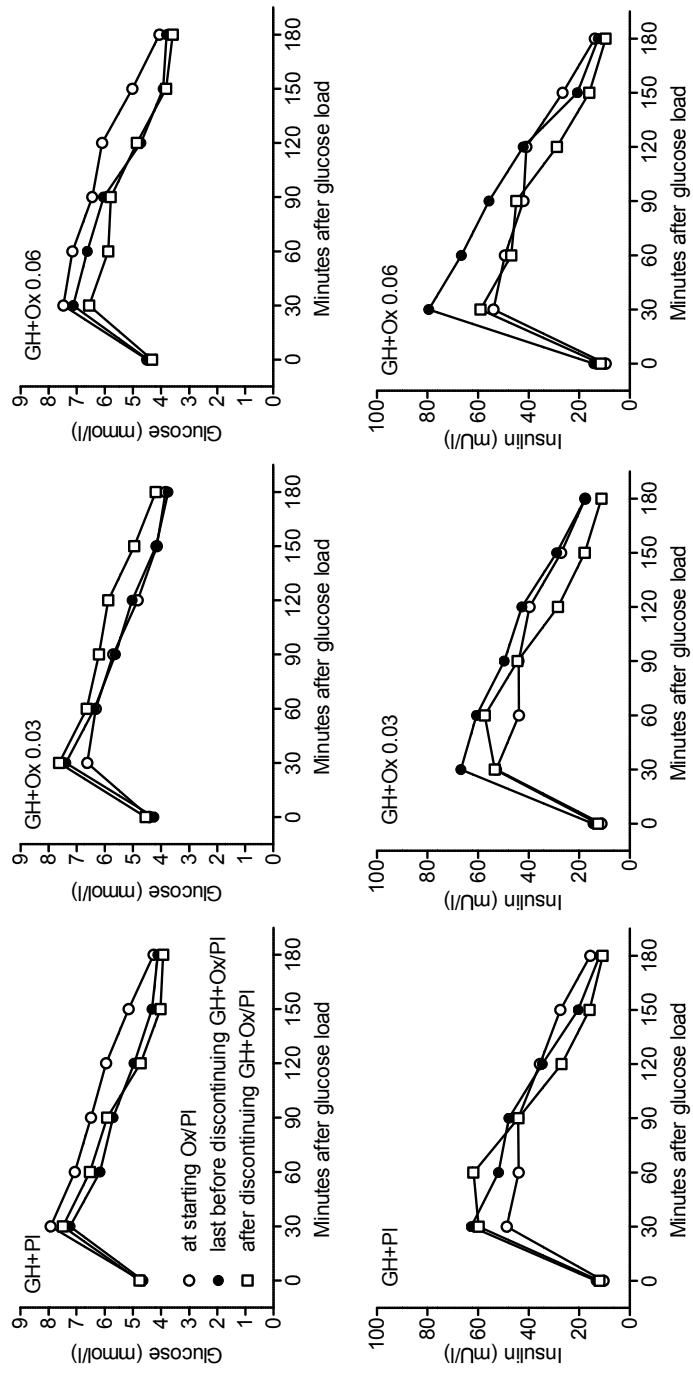

Figure 1 Mean serum glucose and insulin levels during the OGTT.
GH, growth hormone; Ox/PI, oxandrolone/placebo. Note that at starting Ox/PI, patients from age group 1 (approximately a third of the patients) had already been treated with GH. To convert glucose concentration to mg/dl, divide by 0.0555; to convert insulin concentration to pmol/l, multiply by 6.89.

Table 2. Outcome variables per dosage group.*

Outcome variable	GH+Pl (n =38)	GH+Ox 0.03 (n =38)	GH+Ox 0.03 vs. GH+Pl *P Value*	GH+Ox 0.06 (n =36)	GH+Ox 0.06 vs. GH+Pl *P Value*
Impaired fasting glucose					
At starting Ox/Pl – no. (%)‡§	2 (6)	2 (5)		2 (6)	
≥ Once during GH+Ox/Pl – no. (%)†	11 (30)	1 (3)	0.001	3 (8)	0.02
Impaired glucose tolerance					
At starting Ox/Pl – no. (%)‡§	2 (6)	1 (3)		6 (17)	
≥ Once during GH+Ox/Pl – no. (%)¶	3 (8)	6 (16)	0.5	5 (14)	0.5
Fasting glucose – mmol/l‖					
At starting Ox/Pl – mmol/l‡	4.7±0.5	4.6±0.7		4.5±0.6	
Change during first 2 yrs of Ox/Pl – /yr, SE‖	0.02, 0.06	-0.19, 0.06	0.01	-0.12, 0.06	0.09
Change during total duration of Ox/Pl – /yr, SE‖	0.00, 0.03	-0.05, 0.03	0.4	0.00, 0.03	0.9
At disc GH+Ox/Pl**	4.6±0.7	4.2±0.5	0.04	4.5±0.9	0.4
Fasting Insulin – mU/l††					
At starting Ox/Pl – mU/l‡	10.4±6.3	11.2±8.5		9.6±3.8	
Change during first 2 yrs of Ox/Pl – /yr, SE**	0.78, 0.76	0.25, 0.76	0.5	2.25, 0.78	0.1
Change during total duration of Ox/Pl – /yr, SE**	0.42, 0.41	0.91, 0.40	0.5	1.09, 0.42	0.5
At disc GH+Ox/Pl**	13.4±5.5	14.6±9.4	0.9	14.3±10.8	0.7
Insulin sensitivity (WBISI)§					
At starting Ox/Pl‡	6.4±3.1	6.4±2.8		6.5±3.0	
Change during first 2 yrs of Ox/Pl – /yr, SE**	-0.39, 0.25	-0.05, 0.26	0.3	-0.80, 0.27	0.3
Change during total duration of Ox/Pl – /yr, SE**	-0.19, 0.12	-0.24, 0.12	0.7	-0.19, 0.13	0.8
At disc GH+Ox/Pl**	5.7±3.3	5.4±2.4	0.8	5.5±2.6	0.7
Insulin resistance (HOMA-IR)					
At starting Ox/Pl‡	1.3±0.7	1.4±1.0		1.2±0.5	
Change during first 2 yrs of Ox/Pl – /yr, SE**	0.10, 0.09	0.02, 0.09	0.4	0.26, 0.09	0.2
Change during total duration of Ox/Pl – /yr, SE**	0.05, 0.05	0.11, 0.05	0.5	0.13, 0.05	0.5
At disc GH+Ox/Pl**	1.7±0.7	1.8±1.1	1.0	1.7±1.3	0.7

Area under the curve ($IAUC_{gluc}$) – mmol/lx180min					
At starting Ox/Pl‡	255±166	248±139		279±194	
Change during first 2 yrs of Ox/Pl – /yr, SE**	-28, 16	8, 16	0.1	-13, 17	0.2
Change during total duration of Ox/Pl – /yr, SE**	-13, 8	-7, 8	0.2	-33, 8	0.2
At disc GH+Ox/Pl**	171±176	221±154	0.5	175±179	0.6
Area under the curve ($IAUC_{insul}$) – mU/lx180min					
At starting Ox/Pl‡	4522±2813	4652±3059		5031±2582	
Change during first 2 yrs of Ox/Pl – /yr, SE**	425, 330	135, 335	0.6	753, 348	1.0
Change during total duration of Ox/Pl – /yr, SE**	6, 148	-33, 149	0.4	36, 155	1.0
At disc GH+Ox/Pl**	4566±3795	5129±4052	0.4	5776±3736	0.7
HbA1c – %					
At starting Ox/Pl‡	4.5±0.5	4.7±0.4		4.6±0.5	
Change during first 2 yrs of Ox/Pl – /yr, SE**	0.02, 0.03	-0.08, 0.03	0.03	-0.13, 0.03	0.001
Change during total duration of Ox/Pl – /yr, SE**	-0.06, 0.01	-0.06, 0.01	0.8	-0.08, 0.02	0.2
At disc GH+Ox/Pl**	4.4±0.4	4.5±0.5	0.5	4.2±0.4	0.1

* Values are expressed as means ±SD, unless otherwise indicated.
† Fasting glucose levels during Ox/Pl therapy were missing in one patient from group GH+Pl.
‡ 'At starting Ox/Pl' denotes at baseline for age group 2 and 3, and a full number of years after starting GH therapy for age group 1.
§ OGTT data at starting Ox/Pl were missing in two patients from group GH+Pl, and the 120' min glucose level was missing in one patient from group GH+Ox 0.03.
¶ The 120` min glucose levels were missing in three patients on GH+Pl, GH+Ox 0.03, and GH+Ox 0.06, respectively.
‖ To convert glucose concentration to mg/dl, divide by 0.0555.
** Values belonging to the visit at which one girl had a 2-hour glucose of 12.2 mmol/l, and an increased HbA1c were omitted from the analysis.
†† To convert insulin concentration to pmol/l, multiply by 6.89.

Fasting insulin and glucose levels

In the patients that had not been treated with GH before the first OGTT was performed (i.e. the patients from age groups 2 and 3 at a mean age of 11.5±2.2 years), mean fasting glucose level was 4.5±0.6 mmol/l, and the median fasting insulin level was 9.1 mU/l (2.6-51) (data not shown).

Fig. 2A shows mean fasting glucose levels before, during, and after discontinuing Ox/Pl therapy for the three age groups combined. During Ox/Pl therapy, the mean glucose level did not change significantly (from 4.6±0.6 to 4.5±0.7 mmol/l, mean change/yr, SE, -0.02, 0.02 mmol/l/yr, P=0.3). Whereas differences during the total duration of Ox/Pl therapy were not significant, fasting glucose levels lowered more on GH+Ox than on GH+Pl during the first two years on Ox/Pl therapy (GH+Ox 0.03, P=0.01; GH+Ox 0.06, P=0.09). Mean fasting glucose at discontinuing GH+Ox/Pl was somewhat lower on GH+Ox 0.03 and 0.06 than on GH+Pl (P=0.04 and P=0.4, respectively) (Table 2). Thereafter, fasting glucose levels remained constant (4.5±0.8 vs. 4.5±0.7 mmol/l, P=0.9), and levels were still somewhat lower in groups GH+Ox 0.03 and 0.06 than in group GH+Pl (GH+Pl, 4.8±1.1 mmol/l; GH+Ox 0.03, 4.4±0.4 mmol/l, P=0.06; GH+Ox 0.06, 4.3±0.5 mmol/l, P=0.02). None of the patients developed fasting glucose levels below 2.7 mmol/l. Values after discontinuing GH+Ox/Pl were comparable with pretreatment levels (4.5±0.9 vs. 4.5±0.6 mmol/l, P=0.6) (age groups 2 and 3).

Fig. 2B shows mean fasting insulin levels before, during, and after discontinuing Ox/Pl therapy. During Ox/Pl therapy, mean fasting insulin levels increased from 10.4±6.5 to 14.0±8.7 mU/l (mean change/yr, SE: 0.80, 0.24 mU/l, P<0.001), without significant differences between the dosage groups (Table 2). After discontinuing GH+Ox/Pl, the levels decreased from 14.0±8.7 to 12.0±7.1 mU/l (P=0.01). Values after discontinuing GH+Ox/Pl were comparable with pretreatment levels (median, range; 8.9, 4.3-29 vs. 9.1, 2.6-51 mU/l, P=0.4) (age groups 2 and 3).

Insulin sensitivity

Before GH+Ox/Pl therapy (age groups 2 and 3), the mean WBISI was 6.4±3.0 (data not shown). Fig. 2C shows the mean WBISI before, during, and after discontinuing Ox/Pl therapy for the three age groups combined. During GH+Ox/Pl therapy, the

WBISI decreased from 6.4±2.9 to 5.5±2.8 (mean change/yr, SE: -0.21, 0.07, P=0.003), without significant differences between the Ox/Pl dosage groups (Table 2). Similarly, differences in the change of HOMA-IR, $IAUC_{gluc}$, and $IAUC_{insul}$ were not significant between the dosage groups (Table 2). Whereas HOMA-IR increased during therapy (P<0.001), $IAUC_{gluc}$, and $IAUC_{insul}$ did not change significantly (P=0.9 and P=0.6, respectively). After discontinuing GH+Ox/Pl, mean WBISI increased from 5.5±2.8 to 6.7±3.4 (P=0.009). The mean WBISI after discontinuing GH+Ox/Pl was comparable with the mean pretreatment level (6.8±3.1 vs. 6.4±3.0, P=0.4) (age groups 2 and 3).

HbA1c

Except for the girl who developed an HbA1c of 7.7% (see *IFG and IGT*), HbA1c levels remained within the normal range. Before starting GH+Ox/Pl therapy (age groups 2 and 3), mean HbA1c was 4.7±0.5% (data not shown). During GH+Ox/Pl therapy, values decreased from 4.6±0.5% to 4.4±0.4% (mean change/yr, SE: -0.07, 0.01, P<0.001) in age groups 1, 2, and 3 combined. During the first two years on Ox/Pl therapy, HbA1c lowered more on GH+Ox than on GH+Pl (GH+Ox 0.03, P=0.03; GH+Ox 0.06, P=0.001), whereas changes during the total duration of Ox/Pl therapy was not significantly different (Table 2). After discontinuing GH+Ox/Pl, the mean HbA1c value further decreased from 4.4±0.4% to 4.3±0.4% (P=0.009). Mean HbA1c after discontinuing GH+Ox/Pl was significantly lower than mean pretreatment HbA1c (4.3±0.4% vs. 4.7±0.5%, P<0.001) (age groups 2 and 3).

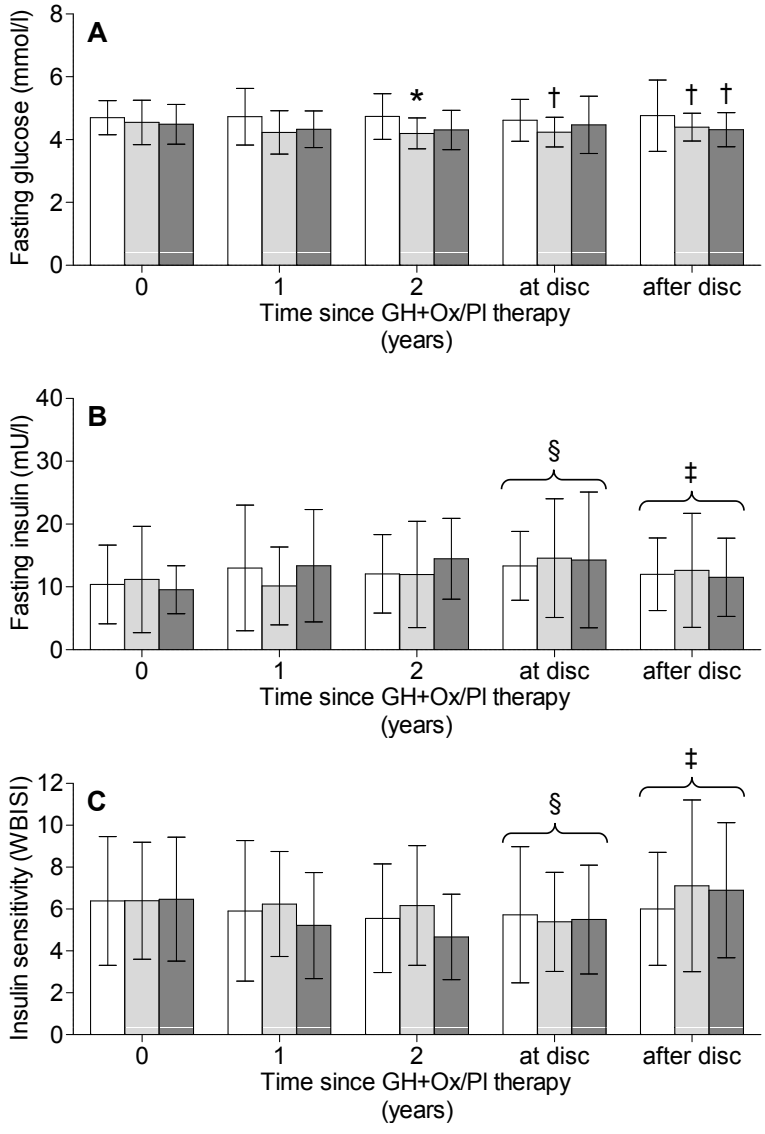

Figure 2 Mean±SD fasting glucose (A), fasting insulin (B), and whole body insulin sensitivity index (C) on GH+Pl (white bars), GH+Ox 0.03 (light grey bars), and GH+Ox 0.06 (dark grey bars). Time point '0' denotes at baseline for age group 2, and 3, and a full number of years after starting GH therapy for age group 1; *, 2 year decrease significantly greater on GH+Ox 0.03 than on GH+Pl; †, at given time-point, value significantly different from that of group

GH+Pl; §, significantly different from values at starting Ox/Pl; ‡, significantly different from values at discontinuing Ox/Pl therapy. To convert glucose concentration to mg/dl, divide by 0.0555; to convert insulin concentration to pmol/l, multiply by 6.89.

Discussion

This randomized, placebo-controlled, double-blind study shows that the addition of Ox at a low (0.03 mg/kg/day) and previously conventional (0.06 mg/kg/day) dosage to GH does not significantly affect insulin sensitivity. In addition, the study demonstrates that insulin sensitivity indices decrease during GH therapy, but return to pre-treatment levels after discontinuing therapy.

Whereas the mean fasting glucose levels were within the normal range and remained constant throughout the study, 21 of the 112 girls (19%) had an impaired fasting glucose at least once. These data may suggest that impaired fasting glucose is a more frequent characteristic in TS than previously acknowledged. In accordance with these data, fasting glucose levels have been reported to be slightly higher in TS women than in age-matched control women [23]. Surprisingly, we found that fasting glucose levels lowered more on GH+Ox than on GH+Pl during the first two years of Ox/Pl therapy, resulting in mean fasting glucose levels that were lower than on GH+Pl at discontinuing therapy. Possibly related to these data, also HbA1c levels lowered more on GH+Ox than on GH+Pl. Although these data remain to be confirmed by other studies, a possible explanation may be sought in the fact that 17-alkylated anabolic steroids related to Ox were found to induce resistance to glucagon in previous studies [10, 24, 25].

We furthermore showed that differences between the Ox/Pl dosage groups in either the percentage of patients developing IGT, or the decrease in insulin sensitivity were not statistically significant. Of the 21 girls (19%) with IGT, seven had IGT before starting GH+Ox/Pl therapy, showing that glucose tolerance may already be impaired in patients as young as 11.5±2.2 years old. Indices of insulin sensitivity (fasting insulin and WBISI) indicated that insulin sensitivity decreased during GH+Ox/Pl therapy. In accordance with our findings, several other studies found that insulin sensitivity decreased during GH therapy [6, 9, 26-28]. These studies showed that

after discontinuing GH therapy, insulin sensitivity indices either returned to pre-treatment levels [27], or decreased to values just above baseline but comparable with those of healthy post-pubertal girls [29]. In our study, the indices returned to pre-treatment levels, confirming the reversibility of the effect of GH on insulin sensitivity. In contrast, no effect [10] and a beneficial effect [30], respectively of GH on insulin sensitivity was found by two other studies. The authors of the latter study suggested that GH administration at the preceding evening may mask positive effects of GH [30]. This is however not in agreement with our findings as we found that GH decreased insulin sensitivity, despite the fact that the girls did not receive a GH dose the night before testing.

Two other investigators have addressed the short-term effect of the addition of Ox to GH on glucose metabolism in girls with TS. Wilson *et al.* compared glucose tolerance in untreated, GH-treated, Ox-treated, and GH+Ox treated girls with TS [10]. Ox at a dosage of 0.125 mg/kg/day, both alone and in combination with GH was found to increase $IAUC_{gluc}$ and $IAUC_{insul}$ in the first year of therapy, whereas fasting glucose and HbA1c remained within the normal range. Haeusler *et al.* showed that in patients who had been treated with GH for one year, the addition of Ox 0.125 mg/kg/day did not change AUC_{gluc}, whereas AUC_{insul} increased significantly in the six months after starting Ox [11]. In contrast to these studies, we did not find an effect of the addition of Ox to GH on insulin sensitivity indices, which suggests that Ox dosages of 0.03 and 0.06 mg/kg/day are low enough to avoid detrimental effects on insulin sensitivity.

We conclude that insulin sensitivity indices decrease during GH therapy but return to pre-treatment levels after discontinuing therapy. In contrast to previously studied higher oxandrolone dosages, no significant side effects with respect to glucose metabolism are expected when adding oxandrolone at a dosage of 0.06 or 0.03 mg/kg/day to GH. Our study furthermore underlines the understanding that monitoring of glucose metabolism is warranted in Turner syndrome, especially during GH or GH+Ox therapy.

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