Endocrine (2012) 41:200–219
DOI 10.1007/s12020-011-9569-8

REVIEW

Sex hormone replacement in Turner syndrome

Christian Trolle · Britta Hjerrild · Line Cleemann · Kristian H. Mortensen · Claus H. Gravholt

Received: 20 October 2011 / Accepted: 12 November 2011 / Published online: 7 December 2011

Abstract The cardinal features of Turner syndrome (TS) are short stature, congenital abnormalities, infertility due to gonadal dysgenesis, with sex hormone insufficiency ensuing from premature ovarian failure, which is involved in lack of proper development of secondary sex characteristics and the frequent osteoporosis seen in Turner syndrome. But sex hormone insufficiency is also involved in the increased cardiovascular risk, state of physical fitness, insulin resistance, body composition, and may play a role in the increased incidence of autoimmunity. Severe morbidity and mortality affects females with Turner syndrome. Recent research emphasizes the need for proper sex hormone replacement therapy (HRT) during the entire lifespan of females with TS and new hypotheses concerning estrogen receptors, genetics and the timing of HRT offers valuable new information. In this review, we will discuss the effects of estrogen and androgen insufficiency as well as the effects of sex HRT on morbidity and mortality with special emphasis on evidence based research and areas needing further studies.

C. Trolle · B. Hjerrild · K. H. Mortensen · C. H. Gravholt (✉)
Department of Endocrinology and Internal Medicine and Medical Research Laboratories, Aarhus University Hospital, 8000 Aarhus C, Denmark
e-mail: ch.gravholt@dadlnet.dk

L. Cleemann
Department of Pediatrics, Hillerød Hospital, 3400 Hillerød, Denmark

K. H. Mortensen
Department of Radiology, Cambridge University Hospitals, Cambridge, UK

C. H. Gravholt
Department of Molecular Medicine, Aarhus University Hospital, 8200 Aarhus N, Denmark

Introduction

Turner syndrome (TS) is caused by a complete or partial absence of one of the X chromosomes and occurs with an incidence of 50 per 100,000 live-born girls [1]. Short stature, congenital abnormalities and estrogen deficiency, due to gonadal dysgenesis, are cardinal features. Affected individuals suffer from increased mortality and morbidity being affected by a broad variety of diseases such as congenital cardio-vascular disease, aortic dilation and dissection, prolonged QTc, metabolic syndrome, atherosclerosis, hypertension, diabetes, hypothyroidism, osteoporosis, bone fractures, cirrhosis, and neuro-cognitive deficits [1–3]. Theoretically, it has been attempted to link the increased morbidity and mortality with ovarian insufficiency as well as X chromosome haploinsufficiency. Unfortunately solid evidence on how and to what extend they contribute are as yet sparse.

In this review, we will present the evidence at hand concerning estrogen and androgen insufficiency and the effect of hormone replacement therapy (HRT) with special emphasis on what is evidence based and in which areas further studies are needed.

The genetic background for premature ovarian failure in Turner syndrome

Early ovarian failure is present in most persons with TS and they hence belong to the heterogeneous group of patients suffering from premature ovarian failure [4]. Until

Table 1 Hormone replacement therapy, proposed dose and route of administration in the different age groups [29]

Age	Proposed treatment	Oral estrogen	Transdermal estrogen
<12	Signs of natural pubertal development. Hormone blood samples (FSH)		
12–13	Absence of spontaneous pubertal development. Elevated FSH	Human estradiol 0.25 mg daily	Plaster of Estradiol 25 μg/day. One quarter of a plaster = 6.25 μg/day
12.5–15	Gradual increase of estrogen dose depending on development	Increase to adult dose (2–4 mg estradiol daily)	Increase to adult dose 100–200 μg daily
14–16	Begin cyclic progesterone treatment after 2 years of estrogen treatment or at breakthrough bleeding	Combined products with estrogen and gestagen, e.g., trisekvens	Transdermal treatment with estrogen is supplemented either with tablets (5–10 mg) 10 days per month or transdermal treatment with progesterone
14–30	Continued hormone treatment at full dose because normal estrogen production is maximum 1–30 years of age	Can consider switching to oral contraceptives (see below)	
30–50	Continued estrogen treatment to counteract risk for osteoporosis and to maintain feminization		
>50	Continued sex hormone treatment depending on risk factors similarly to women undergoing the menopause		

the 18th week of gestation germ cell count in the fetus with TS (45,X) is normal where after a gonadal demise occurs which associates with high levels of FSH and LH as seen in the toddler and early childhood (2–5 years) as well as after onset of puberty (11–12 years) [5]. Levels of FSH and LH are within the normal range during the neonatal period and late childhood [5, 6]. Ovarian failure leads to varying degrees of estrogen, gestagen, and androgen insufficiency [7, 8] with the concern of compromised bone maturation [9–11], osteoporosis and risk of fractures [3, 12, 13], lack of secondary female sex characteristics, underdeveloped uterus [14] rendering oocyte donation difficult [15], infertility, a lack of appropriate cognitive and motor-speed development [16] and cardiovascular disease [3]. The definite cause of the accelerated apoptosis of germ cells in TS is unknown, but one current hypothesis proposes that faulty meiotic pairing triggers the ovarian demise [17]. Only two genes on the X chromosome are clearly implicated in other cases of premature ovarian failure—these are *bone morphogenetic protein 15* (BMP15) and *fragile X mental retardation 1* (FMR1) [18–20]. Other genes have been implicated, but not proven, to have a role in ovarian failure of females with TS [21]. Enhanced understanding of the process of accelerated apoptosis is essential in order to potentially rescue ovarian function in TS and other conditions with premature ovarian failure.

Clinical characteristics and treatment of ovarian failure

As many as 20–30% of TS show signs of puberty and 2–5% have spontaneous menses and they may experience unassisted pregnancy [22–26] and cases of repeated spontaneous pregnancies in 45,X TS has been described [27, 28]. There is international consensus to induce puberty around the age of 12 (Table 1) which coincides with normal puberty in order to avoid social problems related to late pubarche [29]. Raised FSH and LH in the context of absent signs of puberty should lead to commencement of HRT. To simulate normal pubertal development very low doses of estrogen as monotherapy should be titrated guided by the development of Tanner stage, LH, FSH, and bone maturation. Gestagen is added after approximately two years of treatment or when breakthrough bleeding commences. There is lack of research concerning the various types of gestagen and since these compounds have distinct androgenic profiles and also gestagen potency, there is a need to understand potential differences and advantages of specific compounds [30].

Consideration on the dose of estradiol should take into account that prolonged treatment with high doses of estradiol could normalize uterine size and improve the outcome of oocyte donation [15, 31, 32]. A positive correlation between daily estrogen dose and uterine length has been documented [33]. In a cross-sectional study of 41 adolescent, TS females examined by ultrasound and magnetic resonance imaging (MRI), Cleemann et al. [34] showed that uterine volume in relation to breast stage was larger in TS at Tanner-stage B3 but smaller at B5 (Fig. 1), but MRI revealed a lower uterine size across all stages when compared to healthy peers. Ultrasound results are indicative of a dissociation between breast development and uterine growth, which might indicate that the conventional pubertal induction regime is sufficient to induce

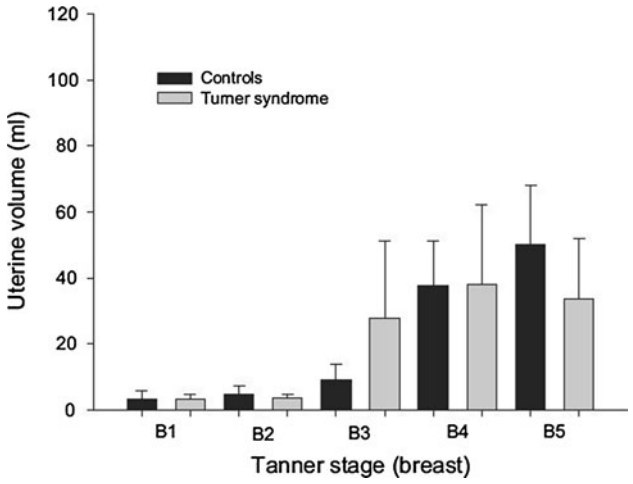

Fig. 1 Uterine volume (ml) by ultrasound in relation to breast developmental stage (Tanner) in Turner syndrome (TS) and controls. Significant *P* values were seen in Tanner stage B3 and B5. The number of individuals in each breast stage TS/controls: B1 = 5/7, B2 = 3/6, B3 11/12, B4 = 11/28, B5 = 11/54. From [34]. With permission by John Wiley and Sons

secondary sexual characteristics, but insufficient in normalizing uterine growth [34, 35].

In the future, it might be possible to combine preparation of the uterus by estrogen in a given TS individual with the subsequent use of autologous oocyte reimplantation saved using cryopreservation at an earlier age [36, 37], though an ethical problem could emerge since the accelerated oocyte apoptosis in TS would indicate that the evacuation of oocytes has to be performed in young girls [36, 37]. Therefore, further studies on how to rescue the primordial follicles from apoptosis, thereby widening the therapeutic window, are merited.

Short stature and sex hormone therapy

Decreased stature affects nearly all females with TS and is present even in the fetus (−1 SD) [38]. The growth stunting increases during infancy and childhood (−2 SD) [38] toward a quite severe reduction around 14 years of age (−4 SD) without recombinant human GH (rhGH) treatment [39–43]. The SHOX gene haploinsufficiency account for 50–75% of the height deficit [44] and associates with a distinct disproportionality in body composition [40]. GH deficiency with reduced GH sensitivity [45–47], and perturbations of the GH–IGF–IGFBP system [48] may also be present and explain why higher doses of rhGH are needed, when compared with other conditions with growth deficiency [49, 50]. This use of higher doses of rhGH leads to elevated circulating insulin-like growth factor I (IGF-I) levels—often in the acromegalic range, and the issue of acromegalic side-effects has been raised [51]. Furthermore evidence supports early introduction of rhGH normalizing height in 93% of toddlers within 2 years [52]. Effects beyond altered bone formation of rhGH in TS include reduced fat mass in addition to an increase in lean body mass, without apparent signs of left ventricular hypertrophy or increase in blood pressure as could be suspected when extrapolating data from acromegalic patients [49]. In the Dutch dose-finding study, concerns were raised about the growth of hands and feet [51] although adult untreated TS females seem to have relatively large hands and feet [40].

It has been discussed if estrogen substitution could limit the effect of growth hormone (epiphyseal closure and enhanced bone mineralization) [53, 54] especially when using orally administered estrogen [55]. Recommendations so far has been to initiate estrogen around the age of expected puberty (12 years) and this approach does not compromise final height [56] (Table 2). Hypothetically the growth stunting could be an estrogen-mediated effect on the GH–IGF-I axis, reducing the IGF-I serum levels and impairing the IGF-I-independent effects of GH on metabolism [57, 58]. One theory propose that growth stunting secondary to HRT could be circumvented by the use of non-orally (i.e., transdermal) administered estrogen [59, 60]. A recent randomized, placebo-controlled, cross-over trial compared the effect of low and high dose micronized 17β-estradiol given for 2 weeks either orally or transdermally with a 2 week washout period interposed. 10 TS (13–20 years) and 20 healthy, age-matched controls participated. Oral 17β-estradiol undergoes first past metabolism and as expected the concentration of estrone (E_1) following oral administration was significantly greater in both the high and low oral dose group when compared to the transdermal group. With respect to LH and FSH, there was no difference in the effect of transdermal and oral high dose but transdermal low dose resulted in a significant greater LH and FSH reduction. No dose regimens normalized FSH and LH and one could therefore argue that the dosing was physiologically insufficient. Interestingly, there was no significant effect on IGF-I concentrations in either the oral or transdermal group [61], similar to findings in a study of adult TS given either oral or transdermal 17β-estradiol [62]. Other studies have seen different effects of oral and transdermal administered estrogen which may be related to higher concentration of estrone after oral administration (first pass metabolism) since estrone has been shown to be an G-protein-coupled estrogen receptor 1 antagonist at high concentrations [63]. The G-protein-coupled estrogen receptor signals as an intracellular non-genomic mediator of some of the effects elicited by 17β-estradiol and other estrogens.

Table 2 Prospective studies on combined therapy with growth hormone and estrogen with final height as endpoint

Topic	Outcome	Reference	Design	Cohort	Age (±SD)	Results	Conclusion
E (childhood low dose) + GH	FH (SD)	Ross et al. [66]	RCT	149 TS A: Double Pl B: E + Pl C: GH (0.1 mg x 3/week) + Pl D: E + GH	A: 7.5 ± 2.3 years of age B: 8.5 ± 2.7 years of age C: 8.2 ± 2.6 years of age D: 9.3 ± 2.5 years of age	A: FH in SD −2.81 ± 0.85 B: FH in SD −3.39 ± 0.74 C: FH in SD −2.29 ± 1.10 D: FH in SD−2.10 ± 1.02 P=0.059	No negative effect of childhood low dose ethinyl estradiol
E + GH	FH (SD)	Chernausek et al. [55]	RCT No Pl	60 TS GH (0.375 mg/kg/week) A: 15 years at E start B: 12 years at E start	A: 9.4 ± 0.9 years of age B: 9.6 ± 1.0 years of age	A: AHG 8.4 ± 4.3 cm B: AHG 5.1 ± 3.6 cm $P<0.01$	A negative effect of early conjugated estrogen on AHG
E + GH	FH (SD)	Van Pareren et al. [56]	RCT No Pl	60 TS A: GH (4 IU/m^2 day) B: GH (first year 4 IU/m^2 day; thereafter 6 IU/m^2 day) C: GH (first year 4 IU/m^2 day; second year, 6 IU/m^2 day; thereafter, 8 IU/m^2 day)	A: 6.5 ± 1.9years of age B: 6.9 ± 2.3 years of age C: 6.9 ± 2.4 years of age	A: FH in SD −1.6 ± 1.0 cm B: FH in SD −0.7 ± 1.0 cm C: FH in SD −0.6 ± 1.0 cm $P<0.01$ $P<0.001$	No negative effect of micronized 17β-estradiol added at 12.7 years of age
E (low dose) + GH	FH (SD) BA/CA-ratio	Quigley et al. [53]	RCT + Pl	232 TS A: GH (0.27 mg/kg/week) + Pl B: GH (0.27 mg/kg/week)+ E C: GH (0.36mg/kg/week) + Pl D: GH (0.36mg/kg/week) + E E: Double placebo	A: 9.7 ± 2.7 years of age B: 9.6 ± 2.7yearsof age C: 9.8 ± 2.9 years of age D: 9.9 ± 2.9 years of age E: 9.4 ± 2.7 years of age	No significant difference in FH	No significant effect of ethinyl estradiol on near FH. Significant increase in BA/CA-ratio Study closed before planned

FH final height, *CA* chronological age, *BA* bone age, *GH* growth hormone, *E* estradiol, *NS* non-significant , PI *placebo*, *SD* standard deviations, *AHG* average height gain

Estrogen may potentially stunt bone growth, still low dose estrogen introduced early on is appealing since it may be beneficial in optimizing peak bone mass, thus prevent osteoporosis in the long term [54] and may improve non-verbal speed processing [64], and non-verbal memory [65]. Especially since the results of studies on the growth stunting effect of orally administered estrogen are ambiguous [66]. Furthermore very low dose estrogen may actually increase IGF-I [67], which together with the potential adverse effects with growth retardation of higher estrogen doses point toward dichotomous effects of estrogens on the GH–IGF axis—a stimulatory effect of low doses and an inhibitory effect of high doses [68–71] although this inhibitory effect has hitherto not been demonstrated in TS [62]. A recent study examined the effect of early instituted oral low dose estrogen treatment on height in randomized placebo-controlled setting. 149 girls (age 5.0–12.5 years) were studied and an equivalent effect of rhGH + placebo and rhGH + estrogen was found, with these two treatment arms being superior to placebo ($P < 0.001$) [66]. Still some peculiarities should be kept in mind. Information on final adult height was lacking in 39% and final height gain was on average less than reported in previous studies. The latter possibly because of the suboptimal rhGH dosing and long dosing interval (0.1 mg thrice a week) used [66].

In conclusion to compensate the GH deficiency and decreased sensitivity rhGH should be introduced at an early stage and though resulting in IGF-I levels within the acromegalic range, the treatment is apparently without acromegalic side effects and have additional positive treatment effects with reduced fat mass and increased lean body mass. 17β-estradiol can be introduced at the age of 12 without compromising bone growth but further studies are merited to establish the optimal route of delivery and the possible effect of early low dose estrogen.

Neuro-cognitive development and the influence estrogen and androgen

Under normal circumstances girls produce minute amounts of estrogen before puberty, excluding an increased estrogen production in the first year of life, with the ovaries as the main source [72]. Animal studies have shown an effect of estrogen on different areas of the brain [73] and it could be that low dose childhood estrogen might improve some of the neuro-cognitive deficits seen in TS which do not improve with HRT initiated at expected puberty, though an effect of X-chromosome haploinsufficiency yet to be identified genes cannot be ruled out as another possible explanation.

An abnormal neuro-cognitive profile is well-described in females with TS. Compared to their healthy peers matched for age, height, IQ and socioeconomic status, non-verbal skills are more often impaired and lower performance IQ manifests as poor arithmetic skills, visuospatial and executive function deficits as well as visual–spatial organization deficits, difficulty with social cognition, problem solving and motor deficits [29].

Ovarian insufficiency and resultant lack of estrogen may potentially be responsible for a part of the neuro-cognitive abnormalities. Studies in animal models proposed that

estrogen could function transitorily as a neuromodulator by modifying the uptake of neurotransmitters or altering neuronal electrical activity or permanently by altering synapse formation and remodeling or both [73]. Estrogen treatment apparently improves executive ability, memory, and motor function in TS [64, 74] though visual–spatial processing, visual memory and arithmetic skills might not improve [64, 74–76]. Beneficial effects of estrogen on neuro-cognitive aspects are further supported by studies of postmenopausal women [77–79] where the effect probably is mediated by non-genomic interaction with the G-protein-coupled estrogen receptor 1 [63]. Recently a randomized, controlled, double-blind trial, examined the effect of low dose synthetic ethinyl estrogen (25 ng/kg/day), with placebo for duration of 1 to 3 years on verbal and non-verbal tasks in TS aged 7–9 years and age-matched female controls. Here a beneficial effect of estrogen replacement was found on the digit span backward test and immediate and delayed recall. Interestingly, verbal and non-verbal tasks were handled similarly in the estrogen treated group of TS girls and the control group [65].

Androgen insufficiency may also affect the neuro-cognitive profile of TS. Males generally perform better than females on spatial perception, spatial visualization, mathematics, and problem solving. Moreover, higher testosterone levels in women are generally correlated with superior spatial abilities. Furthermore, girls with androgen excess due to congenital adrenal hyperplasia have superior spatial abilities compared to siblings, and women treated with androgen and estrogen after ovariectomy have improved complex information processing, logical reasoning and memory [80]. A randomized controlled trial treated 64 girls with TS (51 completed) age 10–14.9 years with oral oxandrolone (0.06 mg/kg/day) or placebo for 2 years in order to test the effect on verbal abilities, spatial cognition, executive function, and working memory. Only working memory improved ($P < 0.03$) but remained minimally impaired [81]. In a 2 year extension of the same trial ($n = 44$) a small decrease in severe learning disabilities ($P = 0.02$) was seen with improved multiplication and division abilities ($P < 0.01$). However results should be interpreted with care since only 58% of participants completed this study [82]. Several factors could contribute to the apparent lack of effect of oxandrolone on verbal abilities, spatial cognition, and executive function including aspects related to the dose of oxandrolone, treatment duration, the fact that oxandrolone and not testosterone or another natural androgen was used, and timing of initiation not to mention a type-2 error due to sample size.

The effect of oxandrolone on behavior, aggression, romantic and sexual interest, mood, and gender role has been examined in a placebo-controlled, double-blind study in 133 TS girls (2–15 years of age) randomized to three groups: rhGH + Ox 0.03 mg/kg/day, rhGH + Ox 0.06 mg/kg/day and rhGH + placebo. Only the externalizing problem *T* score (as tested with child behavior check list) was normalized with no significant difference between the dosage groups and without evident sign of psychological virilizing side effects [83].

In conclusion the neuro-cognitive deficits are presumably related to an interaction between the underlying genetic abnormality (i.e., SHOX or other genes) and the effects of sex hormone deficiency. Estrogen undoubtedly plays an important role and future studies on the effect of early low dose estrogen could possible shed further light on this area. A theoretically basis for the effect of androgens seems plausible but additional studies are merited.

Androgen insufficiency

In females with TS the serum levels of androstendione, dihydrotestosterone, free testosterone, and total testosterone are reduced which is in keeping with more than half of a females testosterone being produced by the ovaries [7, 8]. In normal sexual maturation adrenarche is independent of the ovarian function contrary to the pubertal increase in androgens where the ovaries are the principal source [7]. Therefore, it is of interest to discuss whether androgen replacement therapy should become the mainstay of treatment for females with TS. The potential benefits with androgen substitution in TS could be reduced sexual problems [84], minimization of arithmetic learning disabilities [82], increased bone mineral density (BMD), improved body habitus, working memory and maybe increased height [85–92] though results on the latter are equivocal [93]. Androgens might oppose HRT induced increases in sex-hormone binding globulin (SHBG) levels and hence oppose the reduction in circulating androgens [8]. The precise way androgens stimulate bone growth is still uncertain and one should probably distinguish between the effect of androgens which can be aromatized to estrogen and those that are not aromatized. Oxandrolone is non-aromatizable and does not seem to work through increased GH secretion but via a direct stimulating effect on IGF-I. However, a possible effect on the IGF-I to IGFBP-3 ratio, as an indicator of free IGF-I, has shown conflicting results [94, 95].

Adverse effects of androgens are a concern and include accelerated skeletal maturation, virilization, increased dyslipidemia and an increased insulin resistance [94] though the latter was not confirmed in a recent randomized controlled trial [96].

With GH and estrogen replacement treatment strategies constantly changing over recent decades, it is difficult to compare results of androgen substitution (Table 3). Three

Table 3 Studies on oxandrolone and height gain

Topic	Outcome	Reference	Design	Cohort	Age	Results	Conclusion
Ox (0.05 mg/kg/day) + Early or late E	FH	Gault et al. [92]	RCT + Pl	106 TS. GH (10mg/m^2/week). Ox or Pl from 9 years of age. Ethinyl estradiol from 12 or 14 years of age	7–13 years.	Ox vs. P: ΔFH 4.6 cm (P = 0.001) Early vs. Late E: ΔFH 3.8 cm (P = 0.05) (Ox + early E) vs. Late E: ΔFH 0.7 cm (NS) (Ox + early E) vs. Late E + P: ΔFH(NS)	Significant effect of Ox on final height. No additive effect of late E
Ox (0.06 mg/kg/day)	FH, Lipid, BMD, Tanner, BA	Zeger et al. [97]	RCT + Pl	76 TS. GH (0.35 mg/kg/week). Ethinyl estradiol after 2 years	10–14.9 years	Ox + GH: FH gain 26.2 ± 6.7 cm Pl + GH: FH gain 22.2 ± 5.1 cm P < 0.001	Effect of Ox on final height
Ox (0.06/0.03 mg/kg/day)	Insulin sensitivity (WBISI), SH, Upper arm muscle area, AHG	Menke et al. [95, 96, 100]	RCT + Pl	133 TS GH (1.33 mg/m^2/day). Ethinyl estradiol or 17β-estradiol form 12 years of age. Ox from 8 years of age	Three age groups 1. (2–7.99) 2. (8–11.99) 3. (12–15.99)	GH + Ox (0.03): AHG 9.5 ± 4.7cm GH + Pl : AHG 7.2 ± 4.0 cm GH + Ox (0.06): AHG 8.3 ± 4.7 cm P = 0.3	Significant effect of Ox (0.03) on AHG. Insignificant when correcting for bone age at starting GH therapy
Ox (0.1 mg/kg/day) + late E	Gain over PAH	Stahnke et al. [85]		91 TS, GH given daily, E at 14.9 years A: GH (0.2 mg/kg/week). B: GH (0.2 mg/kg/week) + Ox. C. Transiently GH + Ox	10.3 ± 2.3 years	A: Gain over PAH 3.6 ± 2.6 cm B: Gain over PAH 7.9 ± 3.8 cm C: Gain over PAH 6.4 ± 3.5 cm P < 0.01 NS	Significant effect of Ox on gain over PAH
Ox (0.125 mg/kg/day) + late E	FH	Rosenfeld et al. [87]		70 TS (60 completed, 76 % 45,X) A: Retrospective controls B: Ox C: GH 0.125 mg/kg/3 times per week D: GH + Ox Conjugated estrogen added at 14 years of age	4.7–12.4 years	A: 144.2 cm B: 151.0 cm C: 150.4 cm; Gain over PAH 8.4 ± 4.4 cm D: 152.1 cm; Gain over PAH 10.3 ± 4.7 cm P = 0.019	Significant effect of Ox in combination with GH on gain over PAH
Ox (0.05 mg/kg/day)	Gain over PAH calculated from FH	Nilsson et al. [88]		45 TS A: Ox alone for 1 year then GH added ± E B: GH 0.1 IU/kg/d + Ox C: GH + Ox + E Ethinyl estradiol 100ng/kg/day	9–16 years	A - E: FH: 151.0 ± 6.7 cm: Gain over PAH 7.4 cm A + E: FH: 151.1 ± 5.4 cm: Gain over PAH 4.3 cm B: FH 154.2 ± 6.6 cm: Gain over PAH 8.5 cm C: FH 151.1 ± 4.6 cm: Gain over PAH 3.0 cm P <0.001	Ox accelerates height gain. Early addition of E decelerates height velocity and reduces height gain

FH final height, *CA* chronological age, *BA* bone age, *GH* growth hormone, *E* Estradiol, *NS* non-significant, *PI* placebo, *Ox* oxandrolone, *SH* sitting height, *AHG* adult height gain, *HV* height velocity, *PAH* projected adult height

recent randomized placebo-controlled trials, however, have assessed comparable doses of rhGH, ethinyl estradiol and variable doses of oxandrolone and all reported an additional small height gain when adding oxandrolone to rhGH therapy [92, 95, 97]. Interestingly, there was no additive effect when combining oxandrolone and late initiation of estradiol, and the individual effect of the two treatments was comparable [92]. Despite important differences in design, these studies are in line with earlier findings [85, 87, 88], except supporting a much lower oxandrolone dose (around 0.03 mg/kg/day) mainly because of side effects and a possible accelerated bone maturation exceeding the growth promoting effect [95]. Furthermore, no evidence supports initiation of oxandrolone below the age of 8 or the use of oxandrolone unopposed by rhGH or in combination with early low dose estrogen before the age of 12.

Only one study addresses androgen substitution in adults with TS. This crossover pilot study included 14 TS, age 17–27 years who were randomized to methyl testosterone 1.5 mg or placebo for 1 year each. Methyl testosterone can be aromatized to estrogens and thus, some of the results of the trial could be mediated via an increase in estrogens. Nevertheless, results showed an increased BMD at spine and femoral region, significant reduction in total cholesterol, triglycerides and HDL, increased lean body mass but no change in visceral fat. There was no effect on the visual spatial function but a small improvement in quality of life [98].

In summary, current evidence supports that oxandrolone in doses ~0.03 mg/kg/day and introduced after the age of 8 as an adjunct to rhGH increases height gain at the expense of slower breast development and some risk of virilization. Hirsutism and clitoromegaly revert when oxandrolone is discontinued but voice deepening is irreversible [99]. Potential advantages beyond height gain that require further attention are developments in BMD, muscle mass [100], fat distribution, and improved cognitive function. We need more information regarding the effect of androgen deficiency and replacement in TS on insulin resistance and lipid profile. Finally and certainly not less important, effects of androgen replacement therapy on quality of life would be interesting, both when used as an adjunct to rhGH treatment, but also used during adult life.

Bone mass, fracture risk, and hormone replacement therapy

BMD depends on genetic background, physical activity, diet, local growth factors, and several hormones. In TS, the marked deficiency of estradiol secretion is a concern in terms of not achieving the hormonal stimulation that is pivotal for normal bone mineralization [9–11] and the risk of fractures [3, 12, 13, 101–103] and osteoporosis is increased [3, 13, 101, 104–106]. A fact supported by the finding that patients with spontaneous menstruation have normal BMD in contrast to patients who requires HRT [107, 108]. During normal adolescence there is a marked increase in bone mass accrual which is critical to achievement of optimal peak bone mass, a key determinant of future bone health and low risk of fractures [109, 110].

When addressing the issue of BMD, it is crucial to factor in that conventional areal BMD (aBMD) in TS underestimates the actual BMD. This happens because BMD is measured as a two-dimensional area with the current methodology (dual-X-ray absorptiometry (DXA) scanning) where reduced height leads to a relative underestimation of the real BMD. Instead a three-dimensional volumetric BMD (vBMD) should be the preferred measure wherever feasible [13, 101, 111–116] with a perspective for quantification of vBMD using modalities such as high resolution peripheral quantitative computed tomography in the future.

HRT is crucial to both reach maximal peak bone mass in TS during adolescents and early adulthood [117–119] as well as in later adulthood avoiding a rapid decrease in BMD [106]. Results at hand concerning vBMD in prepubertal girls with TS are unequivocal but the between study comparability is compromised by different methods for BMD measurement, assessment of different skeletal regions, different types of bone (i.e., cortical versus trabecular bone) and other methodological issues such as the use of suboptimal control groups. Normal vBMD has been reported in the left hand and index finger [120], femoral neck [121] as opposed to subnormal levels at the radius [122]. In addition vBMD has been found to be lower in fracture patients [122]. Clarification of the optimum mode for stimulation of bone mineralization, measurement and comparison is cornerstone since recent studies have shown that the risk of fractures is increased even in prepubertal girls with TS [3, 102]. If vBMD is normal in the prepubertal period then the predisposition to fractures might be due to other factors than estrogen deficiency.

In the pubertal group of TS reports on vBMD again relies on the region of measurement with low BMD at the radius, which has been hypothesized to be due to a high content of cortical bone [122]. Contrastingly, normal vBMD has been reported at the level of femoral neck and lumbar spine, consisting largely of trabecular bone [119, 121] (Table 4).

Prepubertal, pubertal, and post-pubertal TS girls have low vBMD [122] that is influenced by the duration of estrogen and rhGH therapy [122]. It is noteworthy that treatment with rhGH without HRT in children and adolescents with TS may improve cortical as well as trabecular vBMD [120].

Recent studies, in contrast to older ones [123], finds that the relative risk of fractures is increased at all ages [3] in TS ranging from a relative risk of 1.25–2.16 [3, 12, 13, 101, 102, 112, 124, 125] occurring especially in the forearm [12, 102, 122]. It is not known if the increased fracture risk can be explained by low levels of total, cortical, or trabecular vBMD, skeletal size, an increased tendency to fall perhaps due to clumsiness, lower muscle mass or other factors still not accounted for. A recent study has found decreased cortical vBMD at the radius in pre-, pubertal and post-pubertal females with TS with a decreased cortical area and a thinned cortex [122]. Computed tomography has recently been used to determine cortical bone area, cross-sectional area, and BMD of the lumbar spine and femur, showing that BMD was significantly lower in TS and negatively correlated with age. Femoral cortical BMD was similar to controls but femoral cross-sectional area as a measure of cortical thickness was significantly decreased [126]. A finding supported by a quantitative sonography study of cortical bone in adults with TS where vBMD was comparable to age, gender, and height matched controls but cortical bone strength was lower in TS [103]. In light of the aforementioned methodological issues, the thinned bone cortex has been proposed to cause falsely low cortical vBMD values with the main effect of TS on bone being related to bone geometry rather than tissue density [127]. Though the findings are divergent, prepubertal girls with TS have been found to have normal cortical vBMD and therefore the cortical bone loss may still be due to ovarian hormone deficiency [102, 120]. However, proper HRT seemingly does not correct cortical BMD when low levels have been established [104, 128]. The thin cortical bone could contribute to the fracture risk in TS which is substantiated by the fact that puberty in normal children represent a peak in the risk of fractures, that half of the fractures are located at the forearm [54] and that children without TS have a thin cortical bone. In children without TS, the thin cortical shell is thought to be related to elevated parathyroid hormone levels in early childhood [54]. Interestingly we have previously documented that serum parathyroid hormone, perhaps due to low 25-hydroxy-vitamin D levels (secondary hyperparathyroidism), is increased in TS [105], though others have not shown a relation between low cortical BMD and parathyroid hormone [104].

Table 4 HRT and the effect on BMD and fracture risk in children and adolescents

Outcome	Method	Location	Reference	Design	Cohort	Results
CBA, CSA, BD	CT prior to GH	LS Femoral	Pitukcheewanont et al. [126]	Retrospective cross-sectional chart-review	22 TS (5–18 years of age) No GH, E or Ox Controls: 22 healthy, age, sex and ethnicity matched	Significant negative correlation between lumbar BD and age Lumbar BD significant lower in TS. Femoral cortical BD similar Femoral CSA lower in TS but not when correcting for BA
aBMD, BA fracture risk	SPA DEXA	Wrist LS	Ross et al. [102]	Case–control	78 TS (4–13 years of age) GH, E, Ox discontinued for 6 months prior to study Controls: 28 controls matched for age, BA, BMI or HA	TS significant higher incidence of wrist fracture Forearm: In TS aBMD was normal for HA and BA, else decreased aBMD Lumbar spine: aBMD (NS)
aBMD		Forearm	Mora et al. [117]	No GH or Ox Case–control	36 TS (10.82 years ± 3.45) A: 16 TS E before age 12 B: 11 TS E after age 12 C: 9 TS with follow-up	Significant higher aBMD in group A Significant increase in aBMD over time in group C but not normalized
vBMD increment	DEXA	Left hand index finger	Sas et al. [120]	Randomized No placebo-controlled	68 TS (2–11 years of age) A: GH 4 IU/m^2/day B: 1. Year 4 IU/m^2/day, then 6 IU/m^2/day C: 1. year 4 IU/m^2/day. 2. year 6 IU/m^2/day, then 8 IU/m^2/day. All groups 17B-estradiol after at least 4 years of GH or age 12	Baseline: vBMD normal Significant increase in vBMD50% in group C Predominantly cortical bone increase No effect of spontaneous puberty on vBMD
vBMD, TB BMC, LTM, BMC/LTM	DEXA	Total body LS FN	Högler et al. [121]	Cross-sectional, longitudinal	83 TS (4–24 years of age) GH, E and Ox according to regional guidelines 51 Pre-, 16 pubertal 16 post-menarchal, 17 longitudinal follow-up	No difference between prepuberty, puberty and postmenarchal groups in vBMD z scores at LS or FN Prepubertal: Significant height-independent decrease in vBMD z score at the LS.
vBMD	DEXA	Proximal and distal radius	Soucec et al. [122]	Cross-sectional design	67 TS (6–19 years of age) ± GH ± E	Osteopenia present with cortical vBMD z score significantly lower in pre-, pubertal and post-pubertal girls with TS Trabecular vBMD deteriorates during and after puberty vBMD lower in fracture patients vBMD correlated to GH and E therapy The main effect of TS on bone is related to bone geometry and not tissue density

Table 4 continued

Outcome	Method	Location	Reference	Design	Cohort	Results
vBMD aBMD	DEXA	LS	Bertelloni et al. [119]	Cross-sectional design	26 TS (1.5–25 years of age) Assessed at final height ERT versus ERT + GH	TS on HRT from adolescence show vBMD in normal range. GH affects final height and aBMD but not vBMD

BMC bone mineral content, *aBMD* areal bone mineral density, *vBMD* volumetric bone mineral density, *LTB* lean total body mass, *FH* final height, *BD* bone density, *CBA* cortical bone area, CSA cross-sectional area, *BA* bone age, *HA* height age, *LS* lumbar spine, *FN* femoral neck, *SPA* single photon absorptiometry, *DEXA* dual energy X-ray absorptiometry, *GH* growth hormone, *E* estradiol, *Ox* oxandrolone, *NS* non-significant, *BMD50%* bone mineral density of the part of the phalanx consisting predominantly of cortical bone (~80%)

Apart from parathyroid hormone and sex hormones another hormone involved in bone metabolism namely osteoprotegerin has been found to be lower in prepubertal girls with TS compared to normal age-matched girls. Osteoprotegerin acts as a decoy receptor by binding receptor activator of nuclear factor κB ligand (RANKL) and hence prevents RANKL-induced osteoclastic bone resorption. Estrogen increases osteoprotegerin production and thereby inhibits RANKL-mediated osteoclastogenesis [129]. Studies investigating the effect of HRT in TS subjects are merited to substantiate the observations.

Girls with TS treated with female HRT from adolescence and onwards seem to maintain a vBMD at the lumbar region within the normal range but not when treated with rhGH alone [119] (Table 5). In 60 adults with TS, we found a reduction in vBMD at the spine but not at the femoral neck or forearm [105]. At the latter position aBMD was significantly reduced indicative of the possible importance of the SHOX gene [105]. Adult women with TS treated with oral medroxyprogesterone and higher than usual doses of subcutaneous 17β-estradiol for 3 years gain BMD at lumbar and spinal levels within the normal range pointing toward a possible anabolic effect of high doses of 17β-estradiol on bone [130]. Moreover, conventional doses of HRT maintain BMD at most sites (lumbar, hip, and forearm except radius 1/3 BMD) when followed up of up to 6 years [131]. In addition, we recently reported a parallel increase in aBMD with age and comparable levels of aBMD and vBMD in TS and their peers at the spine and hip while accrual at the cortical part of the forearm was inferior [132].

The mechano-stat theory posits that mechanical forces exerted by muscles deform or strain bones and when straining exceeds a certain "modeling threshold" an increase in remodeling occurs that alters bone mass and architecture [133]. In the light of this the thinner cortical bone in TS may be due to a decreased muscle mass, and secondarily decreased force acting on bones. Especially since it can be expected that short people have proportionally lower muscle forces acting at their skeleton and hence less drive on their periosteal expansion [134]. However, this was not supported in a study of adolescents with TS were the cross-sectional area in the forearm muscles was normal relative to the external bone size and comparable to that of age-matched controls though low cortical thickness was documented in TS [128].

In conclusion, the exact role played by estrogen on BMD in TS and whether a normal peak bone mass is achievable with adequate HRT are awaiting further clarification [101, 106, 114, 115]. Furthermore it is uncertain if HRT can return the risk of fracture to normal. This is especially important in the light of the increased risk of fractures at all ages and that vBMD is normal in most children with TS. Increased risk of fractures and low

Table 5 HRT and the effect on BMD and fracture risk in adults

Topic	Method	Location	Reference	Design	Cohort	Results
BMC cortical thickness	SPA	Forearm	Naeraa et al. [106]	Cross-sectional	50 TS (21–45 years of age) (46 on ERT)	BMC and cortical thickness positively associated with E therapy in TS above 30 years of age
BMC, aBMD, vBMD, IGF-I, T, SHBG, PTH, Vitamin D	DEXA	Forearm LS FN	Gravholt et al. [105]	Cross-sectional, controlled	60 TS (37 ± 9 years of age) 181 matched controls	In TS BMC and aBMD was universally reduced. vBMD only slightly reduced in lumbar region in women with TS Uncoupling of bone resorption and formation suggested
vBMD, effect of high dose sc. E2 + oral progestin	DEXA	LS Proximal femur	Khastgir et al. [130]	Longitudinal, prospective, cohort study, 3 years	21 TS(20–40 years of age) All been on HRT	Osteopenia at baseline vBMD increased significantly Estrogen capable of exerting an anabolic effect on bone Increase in active formation period Suppression of bone turnover
Cortical & trabecular vBMD	DEXA	Forearm	Bakalov et al. [104]	Cross-sectional, controlled	41 TS (18–45 years of age) 35 Controls with premature ovarian failure	Women with TS significantly lower cortical vBMD z score Also when adjusted for height, age of puberty, E, 25-OH-D Independent of testosterone, IGF-1 and PTH
Cortical + aBMD + vBMD	DEXA QUS	Radius Tibia	Zuckerman-Levin et al. [103]	Case–control Ethinyl estradiol	A: 27 TS (21.1 ± 6.3 years of age) B: 53 sex and age-matched controls C: 34 height matched controls	Higher fracture incidence per 1,000 women years in women with TS compared to controls QUS significantly lower in women with TS Difference in vBMD insignificant
Geometry of the FN	DEXA	FN	Nissen et al. [127]	Cross-sectional, controlled	58 TS (22–67 years of age)	Significant different HIP geometry in women with TS Cannot explain increased risk of hip fractures
BMD	DEXA	LS FN Forearm	Cleemann et al. [131]	Prospective observational cohort study Follow-up 5.9 years ± 0.7	54 TS (43 ± 9.95 years of age) HRT (conventional)	BMD can be maintained at most sites Only radius 1/3 aBMD declined
aBMD, vBMD	DEXA	LS FN	Cleemann et al. [132]	Cross-sectional	37 TS (16.7 ± 3.4 years of age) A: On-going GH B: Previously GH	Accrual at the cortical part of the forearm is inferior Evidence of imbalance between formation and resorption in the group not receiving GH Normalization of BMD after GH and continued E at the LS aBMD only reduced in the young and in adults Cortical aBMD was reduced at the forearm

BMC bone mineral content, *aBMD* areal bone mineral density, *vBMD* volumetric bone mineral density, *IGF-I* insulin-like growth factor I, *T* testosterone, *SHBG* sex hormone-binding globulin, *PTH* parathyroid hormone, *SPA* single photon absorptiometry, *E2* micronized 17β-estradiol, *QUS* quantitative ultrasonography, *BA* bone age, *LS* lumbar spine, *FN* femoral neck, *DEXA* dual energy X-ray absorptiometry, *GH* growth hormone, *E* estradiol

vBMD may most certainly be caused by more than one factor, e.g., abnormality in the bone geometry, thin cortical bone, SHOX gene haploinsuffciency, reduced androgen levels [135], perturbed GH/IGF-1 axis, low vitamin D, high parathyroid hormone, an increased tendency to fall [13, 125] or other features associated with TS. Future studies comparing aBMD and vBMD (trabecular and cortical) in TS with other patients affected by SHOX haploinsufficiency may add valuable information.

In a clinical context, estrogen therapy timed as to achieve peak bone mass and inducing puberty without compromising final height, is of utmost importance. Vitamin D, calcium supplements, and regular exercise as well as measures to prevent falls are pivotal.

Glucose metabolism, body composition, and HRT

Fasting glucose level is not significantly elevated in TS when compared to controls, but fasting hyperinsulinemia and impaired glucose tolerance has been found with a frequency varying from 25 to 78% in adults with TS. An explanation seems to be decreased insulin sensitivity and reduced first phase insulin response (a hallmark in the development of type 2 diabetes, T2D) [136–138]. Risk factors of the metabolic syndrome are present in TS with a body composition with increased BMI, decreased muscle mass, elevated total fat mass and visceral fat mass as well as a decreased maximal oxygen uptake (VO_2-max) [62, 139]. A positive family history of T2D is prevalent, but also TS females without this disposition exhibit previous mentioned characteristics [140]. The effect of HRT has been ambiguous. We found an increase in impaired glucose tolerance after OGTT prevalent in 78% after HRT compared with 50% before HRT but that free fat mass and physical fitness increased on HRT [138].

As to route of administration of HRT two recent studies have been unable to document a change in glucose metabolism [61, 137].

In conclusion a large number of TS have inappropriate low levels of insulin and abnormal glucose tolerance both with and without HRT, which might be caused by early beta-cell failure [138, 139, 141, 142].

Liver function and HRT

Elevated liver enzymes has been reported in TS with a prevalence of around 36–80% [143, 144] as wells as a five times increased risk of cirrhosis [3]. The effect of HRT on the liver has been of concern since early reports on postmenopausal women using conjugated estrogen and studies on TS girls receiving 17β-estradiol transdermally or ethinyl estradiol orally, supported a negative effect on hepatic enzymes [145, 146]. However, a study documented a significant reduction in alanine aminotransferase and gamma glutamyl transferase when treating TS adults with 17β-estradiol [62], a finding supported by an un-blinded, randomized, cross-over study [147], a longitudinal study [148] as well as a prospective study [149] all in TS adults. The liver enzyme lowering role of estrogen has also been studied in hypopituitary women supporting the protective effect of estrogen [150] and in a double blinded, randomized placebo-controlled setting in T2D affected adults without TS [151]. Also tamoxifen treatment is associated with steatohepatitis confirming the protective role of estrogen. Interestingly liver function tests in TS are not affected in spite of the elevated liver enzymes [148, 152]. Instead a positive association between total cholesterol and gamma glutamyl transferase in TS has been reported [144].

Roulot et al. assessed the histopathology from liver biopsies in 27 TS with persistently elevated liver enzymes, followed for 8.8 ± 5.2 years. 2 patients had cirrhosis associated with obliterative portal venopathy, 2 multiple focal nodular hyperplasia, 6 nodular regenerative hyperplasia. Less severe changes included portal fibrosis, inflammatory infiltrates and non-alcoholic fatty liver disease. It was concluded that the main cause for elevated liver enzymes was congenital vascular disorders and non-alcoholic fatty liver disease [153]. Interestingly the liver expresses estrogen receptor alpha (ERα) and beta (ERβ), through which estradiol is a direct transcriptional regulator of vascular endothelial growth factor (VEGF), again activating VEGF-receptor 1 and 2 of liver sinusoidal endothelial cells leading to paracrine release of growth and survival factors protecting hepatocytes from toxins and promoting hepatocyte proliferation. A lack of estrogen could in this way lead to accelerated hepatocyte apoptosis and leakage of liver enzymes and vice versa [152].

In a recent study, an abnormality of hepatic lipid storage was suggested since TS women with high cholesterol and high BMI had higher gamma glutamyl transferase and MRI confirmed intra-hepatocellular lipid deposition in TS [148].

In conclusion, there is no evidence of a harmful effect of HRT on the liver but rather a protective effect seems likely maybe through decelerated apoptosis or a normalization of hepatic lipid storage. Further studies are needed to elucidate this area and especially evaluate if the improvement in liver enzymes is just an epiphenomenon or actually will lead to a lesser degree of morbidity related to the liver or if other adverse effect of HRT will predominate.

Physical fitness and HRT

HRT has a positive effect on physical fitness and results in increased muscle mass with improved muscle composition

with lower intra-muscular fat and muscle performance in postmenopausal women [154, 155]. Since TS has a maximal oxygen uptake that is diminished by 25% [136, 153] HRT is appealing and does result in an increase in maximal oxygen uptake with an increased fat free mass [62] though increased physical fitness, muscle mass and fat free mass may also improve through changes in GH/IGF-I, ghrelin, leptin, and insulin sensitivity [156], the self-reported low level of physical activity [62], or the haploinsufficiency of the X chromosome in itself.

New randomized, controlled trials are merited evaluating the effect of intervention with HRT in different doses and in combination regimes with androgens as well as evaluating the different treatments at different ages. It is likely that androgen replacement would also have a positive effect on physical fitness.

Cardiovascular system and HRT

Mortality in TS is increased more than threefold (Fig. 2) and cardiovascular disease accounts for around 50% of the excess mortality observed in TS [1–3]. Trials on the cardio-protective effects of oral HRT as primary and secondary prophylaxis in cardiovascular disease in postmenopausal women has been discouraging without any reduction in cardiovascular risk but an elevated risk of breast cancer, venous thromboembolism, cardiovascular heart disease, and stroke especially when combined with medroxy-progesterone acetate [157]. However, in these studies conjugated equine estrogens were used. Unopposed use of conjugated equine estrogen showed similar results for stroke and venous thromboembolism [158]. Subsequent analyses of these studies have shown a lower risk of these outcomes in younger menopausal women and indicate that age seems to be a critical interactive factor [159]. Additional analyses indicate an increased risk of cardiovascular heart disease during the first 1–6 years of treatment (risk ratio 1.08), but lower risk in year 7 to 8 + (risk ratio 0.46) [160]. Considering that TS females probably should be regarded as a completely different entity of the hypoestrogenic state, benefits, and risks of HRT may be quite different and more likely comparable with that of other populations suffering from premature ovarian failure. HRT could theoretically have a lipid lowering effect, work as an antioxidant [161], interact with endothelial smooth muscle [159, 162], regulate clotting factors [159], have an antagonistic effect on the renin-angiotensin-aldosterone-system [163] as well as the previously mentioned effects on visceral fat, insulin sensitivity, etc. [159], and hence maybe if used in a timely fashion be cardio-protective. Still we lack long-term studies to confirm the cardio-protective effect.

Contributing to the cardiovascular risk profile 30% of girls with TS have a mildly hypertensive 24-h ambulatory blood pressure and 50% are non-dippers [138, 164], which suggests an autonomous neural dysfunction. Consistent with such an autonomous neuropathy is the presence of elevated 24-h heart rates in TS when compared to controls [138]. HRT has been shown to have a positive effect on blood pressure reducing 24-h diastolic pressure significantly [138].

We examined the effect of short-term HRT on the ambulatory arterial stiffness index in TS and found no adverse or beneficial change [165]. Conversely, Ostberg et al. [166] examined the effect of 17β-estradiol on another surrogate measure of cardiovascular risk, the intima media

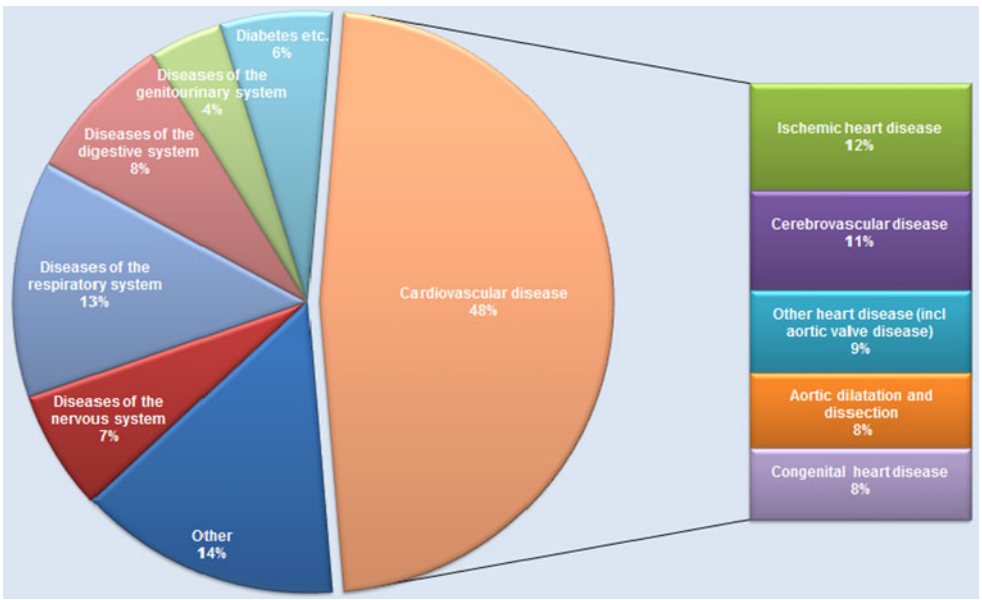

Fig. 2 Absolute excess mortality based on 3,439 women from Great Britain diagnosed with Turner syndrome between 1959 and 2002 with a total of 64.215 observation years. The figure is created based on results from [2]. *Numbers on chart* indicates percent of absolute excess mortality (Other: accidents and violence, congenital anomalies other than cardiovascular anomalies, diseases of the musculoskeletal system and connective tissue, all malignant neoplasms, infectious and parasitic disease)

thickness, in doses of 1, 2, and 4 mg for 12 weeks and found a significant reduction in intima media thickness with higher doses of 17β-estradiol. In addition, they showed that only the high dose of 4 mg of estradiol was able to normalize FSH and LH values, while FSH and LH remained elevated on 1 and 2 mg of estradiol.

It should be mentioned that a prolonged QTc interval is present in as many as 30% of TS women [167] and 20% of TS girls and adolescents [168]. This is interesting since recent studies found a negative correlation between serum testosterone and QTc length [169, 170] and that testosterone could protect against drug-induced arrhythmia [171]. Maybe androgen substitution in order to improve growth and BMD could also have an effect on QTc and risk of sudden death.

In conclusion, the jury is out as to whether HRT might have a cardio-protective effect in TS when started timely as well as concerning what dose and route of administration to prefer and long-term studies with hard endpoint are crucial.

Autoimmunity and HRT

Autoimmunity in TS is increased at least twofold [3, 172] with autoantibody positivity seen in 58% [173]. Both female and male-predominant autoimmune diseases are increased, though the latter is more prevalent [172]. The imbalance or haploinsuffiency of X chromosome material [174] has been proposed as a possible explanation, though lack of estrogen and up-regulation of pro-inflammatory cytokines [156] might also be involved.

Estrogen has numerous effects on the immune system encompassing development of T cells [175] and inhibition of inflammation [176] though estrogens role in autoimmune disease is still uncertain. Studies in human and mice are inconclusive. At periovulatory to pregnancy levels 17β-estradiol has shown a stimulatory effect on IL-4, IL-10, and IFN-γ but inhibition of TNF from CD4+ T cells indicating a shift toward down regulation of T cell autoimmunity [176]. At the same levels 17β-estradiol has shown to stimulate antibody secretion by CD5 + B cells but suppress the bone marrow B cell lineage precursors [176].

In conclusion the exact role of estrogen in autoimmunity in TS is unknown. Current studies indicate that estrogen could induce an increased prevalence of B cell autoimmunity but a decreased *T* cell autoimmunity [176]. Other causes for the breakdown of self-tolerance such as fetal microchimerism, skewing of X chromosome inactivation, gene duplication or up-regulation of pro-inflammatory cytokines are being investigated. Androgen exerts a predominantly inhibitory effect on the immune system [176] and could therefore also be implicated since TS is a relative androgen insufficient state.

Quality of life and HRT

Studies of quality of life in TS are few. A recent review [29] concluded that adult height does not appear to impact quality of life in adulthood, which is in agreement with a long-term randomized controlled trial [177] and the fact that individuals receiving current medical care have normal self-reported quality of life [29]. The latter is in discordance with a small study on 18 girls with TS that reported quality of life to be inferior to that of age- and sex-matched controls [178]. A larger Swedish study ($n = 111$) in adults with TS found that quality of life was negatively affected by older age and advanced age at diagnosis, while positively affected by better body balance, fine motor function, and higher BMD with no aspects of quality of life attributable to rhGH treatment [179]. However, a recent Cochrane review concluded that girls treated with rhGH have better psychological adjustment than untreated girls [180]. Knowledge of the impact of HRT on quality of life is lacking. In conclusion, long-term studies with quality of life as main outcome measure are warranted.

Clinical treatment

Estrogen deficiency should be treated (Table 1). Initiation at the age of 12 does not seem to affect final height. But there are uncertainties as to whether earlier initiation of low dose estrogen during childhood, mimicking the normal physiological levels, could actually have a positive effect on not just final height but also vBMD, fracture risk, neurocognitive development, muscle strength, physical fitness, the cardio-vascular risk profile and quality of life. In view of the timing hypothesis [159] studies within this area are crucial. Evidence concerning the type of estrogen is equivocal but natural 17β-estradiol is the preferred choice and that is what we recommend. Preparations with conjugated equine estradiol should be avoided, since it contains more than 100 estrogenic compounds of different estrogenic potency [181] which cannot be measured reliably in any assay [182]. The preferable route of administration has not been determined, although transdermal administration could offer a more physiologic delivery, while studies performed so far has not shown any consistent differences in TS [8, 58, 60–62, 145, 146, 183–185]. Normally HRT is given in the morning, but one study supports evening administration of HRT since timing of estradiol modulates insulin, IGF-I and glucagon favorably during ongoing rhGH treatment in adolescent TS girls [186], but more studies are necessary to elucidate whether there are advantages to evening administration in adults. Present regimens of HRT appear to maintain vBMD but still not normalize cortical vBMD nor fracture risk. Conventional

Box 1 Suggested clinical out-patient program for patients with Turner syndrome

Baseline	Karyotype
	Renal and pelvic ultrasound
	Echocardiography
	Thyroid status and antibodies
	Celiac screen
	Gonadotropins
	Renal and liver function
	Bone densitometry (DEXA scan)
	Consider psychologist
Annual	Physical examination, including blood pressure
	Thyroid function
	Body composition status (BMI < 25), including physical exercise and diet
	Instruction
	Fasting lipids
	Fasting blood glucose
	Renal and liver function
Every 3–5 years	Echocardiography, MRI of the aorta
	Bone densitometry (DEXA scan)
	Audiogram
	Celiac screen
	Thyroid antibodies (thyroid peroxidase) and other relevant antibodies

HRT does not normalize uterine size, muscle strength, physical fitness and cardiovascular risk profile nor completely abolish neuro-cognitive deficits. It could be hypothesized that the currently used doses of HRT are insufficient. A higher dose may be desirable, especially since the current dose does not raise concern of increased cancer risk and epidemiologic data supports a lower breast cancer risk in TS [2, 187].

Patients with TS need a comprehensive care preferably from a multidisciplinary team, which can best be practiced from an out-patient clinic with special emphasis on TS (Box 1). Knowledge concerning TS is still limited and most clinicians only see TS patient infrequently, and patients typically have a range of questions related to the syndrome when we first see them.

Glucose metabolism, weight, thyroid function, bone metabolism, blood pressure, liver function, and cardiovascular status should be assessed (Box 1). We recommend a centralization of clinical care. A broad cooperative effort is ideal with involvement of a host of specialties, for example, we enjoy the participation of departments of cardiology, gynecology (including a fertility clinic), otorhinology, ophthalmology, pediatrics, psychology, and gastro-enterology.

Summary

Important advances have been made in the care of TS with international guidelines and standardized multidisciplinary evaluation of adult women with TS being effective in revealing undiagnosed morbidity and optimizing medical care in order to hopefully reduce morbidity and mortality [188]. However, many areas await exploration and it is of utmost importance to elucidate how medical intervention affects not just physical health but also quality of life.

Studies aiming to shed light on the consequences of the haploinsufficiency of the X chromosome, and hence the genetic mechanisms behind the increased morbidity and mortality are necessary and in combination with the increasing knowledge on estrogen deficiency and estrogen receptors this might supply new evidence on how to optimally treat Turner syndrome.

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