Epidemiological, endocrine and metabolic features in Turner syndrome

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Abstract

Turner syndrome is one of the more common genetic disorders, associated with abnormalities of the X chromosome, and occurring in about 50 per 100,000 liveborn girls. Turner syndrome is usually associated with reduced adult height, gonadal dysgenesis and thus insufficient circulating levels of female sex steroids, and infertility. A number of other signs and symptoms are seen more frequently with the syndrome. Morbidity and mortality are increased. The average intellectual performance is within the normal range. A number of recent studies have provided new insights with respect to epidemiology, cardiology, endocrinology and metabolism. Treatment with GH during childhood and adolescence allows a considerable gain in adult height, although very-long-term consequences of this treatment are not clear. Puberty has to be induced in most cases, and female sex hormone replacement therapy is given during the adult years. The proper dose of hormone replacement therapy (HRT) has not been established, and, likewise, benefits and/or drawbacks from HRT have not been thoroughly evaluated. Since the risk of cardiovascular and endocrinological disease is clearly elevated, proper care during adulthood is emphasized. In summary, Turner syndrome is a condition associated with a number of diseases and conditions which are reviewed in the present paper.

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Introduction

Turner syndrome (TS) occurs in about 50 per 100,000 girls, and, as such, is one of the most frequent chromosomal aberrations in females. Turner syndrome was named after Henry H Turner of Oklahoma City, Oklahoma, USA who, in 1938, described several females whom he suggested suffered from one and the same syndrome (1). Others had described females with the syndrome before H H Turner, but were probably unaware of the syndromological nature of the condition (2, 3). The original description focused on infantilism, cubitus valgus and congenital webbing of the neck. Later, a number of additional conditions, characteristics, and abnormalities were described. Turner syndrome has a genetic background, with characteristics involving numerous specialities such as embryology, pediatrics, endocrinology, cardiology, otorhinonology, ophthalmology and epidemiology. In this review the focus is on aspects of epidemiology, endocrinology, metabolism, cardiology and body composition of the syndrome with reference to recent genetic discoveries.

Epidemiology

Diagnosis

Clinical features The designation 'Turner syndrome' is a clinical characterization. Today, no firm guidelines for the diagnosis exist (4), but most agree that the cardinal stigmata include growth retardation with reduced adult height with or without additional phenotypical features, and except in rare cases, also gonadal insufficiency, and infertility. The phenotype must be accompanied by a karyotype with complete or partial absence of one sex chromosome, and in addition, mosaicism with two or more cell-lines may be present. Congenital malformations and conditions often seen in TS are given in Table 1, with tentative frequencies. In utero, increased nuchal fold thickness, or more severely, hydrops formation may be present (5), as well as increased fetal heart rate (6). In cases with increased nuchal fold thickness, transvaginal echocardiography often reveals cardiac malformations (7). The combination of increased nuchal fold thickness on ultrasound examination, increased maternal serum levels...
of pregnancy-related protein A and beta-human chorionic gonadotropin are suggestive of Turner syndrome or of other chromosomal aberrations (5).

Genetics The genetic background for the Turner syndrome phenotype is highly variable but includes anomalies of the sex chromosomes (the X and/or Y chromosomes). The prototypical karyotype of a textbook female with Turner syndrome is 45,X; i.e. one X or one Y chromosome is missing. Statistically, it can be estimated that approximately two-thirds of all Turner patients with the 45,X karyotype should have the 46,XX complement, and one-third the 46,XY complement. Today, however, it is established, however, that most women with Turner syndrome are not carrying the ‘typical’ karyotype of 45,X, but rather several different variants all causing the clinical signs of Turner syndrome. The most frequently occurring karyotypes are 45,X, karyotypes with an isochromosome of X (i(Xq) or i(Xp)), the mosaic karyotype of 45,X/46,XX, and karyotypes containing an entire Y chromosome or parts thereof. A list of the karyotypes found in the Danish Cytogenetic Central Register (DCCR) (8) during 1970 – 2002 is presented in Table 2. The DCCR covers all diagnosed females with Turner syndrome in Denmark, and thus represents a unique tool for studying karyotypical aspects of Turner syndrome, including secular trends, induced abortion rates, prevalence and diagnostic delay. In addition, it offers the opportunity for merging this register with other registries in Denmark. The 45,X karyotype was found in only 47% of all liveborn females with Turner syndrome during the period 1970 – 2002. Some researchers claim that the pure 45,X karyotype does not exist, because such an individual could not survive in utero (9, 10). This claim is supported by meticulous studies examining more than one tissue (i.e. other than lymphocytes) for the presence of mosaicism. The issue of mosaicism, especially low-grade mosaicism, is particularly difficult. How should a person with 5% (or less) 45,X cells and 95% 46,XX cells be considered, knowing that normally a karyotype is performed on

Table 1 Continued.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td>Abnormal positioning or duplication of renal pelvis, ureters or vessels</td>
<td>15</td>
</tr>
<tr>
<td>Renal aplasia</td>
<td>3</td>
</tr>
<tr>
<td>5. Psychosocial problems*</td>
<td></td>
</tr>
<tr>
<td>Emotional immaturity</td>
<td>– 40</td>
</tr>
<tr>
<td>Specific learning problems</td>
<td>– 40</td>
</tr>
<tr>
<td>Mental problems</td>
<td>– 25</td>
</tr>
<tr>
<td>Neurocognitive deficits</td>
<td>?</td>
</tr>
<tr>
<td>6. Others</td>
<td></td>
</tr>
<tr>
<td>Poor thriving during 1st year of life</td>
<td>50</td>
</tr>
</tbody>
</table>

* The data in the literature are inconsistent, and the given percentages should be viewed with caution.
lymphocytes and therefore only represents this tissue? Information regarding the level of mosaicism should probably always be considered as no more than an adjunct to the clinical examination of the fetus or the individual, which eventually determines whether a person should be considered as suffering from Turner syndrome.

Incidence and prevalence

Prenatal studies The prenatal prevalence is much higher than the postnatal prevalence (11). This indicates that a high rate of conception of TS fetuses occurs. This is illustrated by a very high prevalence of Turner syndrome karyotypes after chorion villus sampling (performed on average in week 11) of 392 TS fetuses per 100,000 female fetuses compared with a prevalence after amniocentesis (week 16) of 176 per 100,000 (11). There is a well-described increased intrauterine mortality, especially during the first trimester (peaking in gestational week 13) (12), but after this period there is no or only slightly increased intrauterine mortality (11). Prenatal diagnosis of TS is not always correct, especially when taking into account mosaic cases (11, 13–16), and it is even more complicated in rare cases with twins (17). This means that one should not rely solely on a karyotype before deciding what action to take (legal abortion or continued pregnancy), but rather one should perform a high-resolution ultrasound scan or fetal echocardiography and other modern modalities, in order possibly to diagnose additional features of Turner syndrome (i.e. hygroma, webbing of the neck, increased nuchal translucency, congenital heart defects, horseshoe kidney, bone anomalies, etc.) (5, 18–21). Prenatally ascertained mosaic cases have a more benign course than postnatal ascertained cases, which might be due to postnatal ascertainment bias. Most prenatally diagnosed fetuses with Turner syndrome are legally aborted. This is, of course, a choice made by the parents after consultations with, most commonly, a geneticist or an obstetrician. A recent European multi-center study (19 centers and 11 countries) found an induced abortion rate of 66%, with a higher abortion frequency (79%) when fetuses were identified after ultrasound scans as opposed to fetal karyotyping (42%) (22). The study essentially confirms previous studies showing legal abortion rates of 60–80% (11, 23, 24). So far, no consistent difference across countries is apparent.

Postnatal studies The prevalence of TS is based on a number of cytogenetic studies with estimates ranging from 25–210 per 100,000 females (12, 25–27), and a hypothetical proportion of about 50 per 100,000 girls in Caucasian populations may be agreed upon. Thus, one would expect 17–18 newborns with Turner syndrome every year in Denmark (total population: 5,250,000; female birth rate: approximately 30,000 liveborn) (11), making it one of the most common chromosomal disorders. Currently, however, the diagnosis of TS is made more infrequently than would be expected from the original cytogenetic surveys (11, 28), and a considerable delay in diagnosing girls and adolescents with the syndrome is obvious (28). In Denmark, we found a prevalence of 32 per 100,000 liveborn girls during 1970–1993 (11), leaving some hypothetical 18 per 100,000 liveborn TS patients undiagnosed. However, with time, more TS patients are diagnosed. This is illustrated by updating Danish data on prevalence. When re-assessing prevalence during the years 1970–1993, 10 years later the prevalence had risen to 40 per 100,000 liveborn girls (Fig. 1); this underlines the fact that TS is often diagnosed late, and does not indicate a true increase in prevalence. Likewise, in a North American study of 81 females with TS the average diagnostic delay was 7.7 ± 5.4 years during childhood and adolescence (28). Interestingly, the key to diagnosis was lymphedema in 97% during infancy, and short stature in 82% during childhood and adolescence. Due to the nature of the study (only children) there was no information regarding the key to diagnosis in adults. The study also documented that the vast majority of patients had several stigmata at the time of diagnosis, which would have been expected to facilitate an earlier diagnosis (28). Thus, the delay in diagnosis could not simply be explained by lack of manifestations of TS in this population. In addition to delay in diagnosis of the syndrome during childhood and adolescence, it must be emphasized that TS is also diagnosed in adults. When studying the entire population of females with TS in Denmark,

Table 2 Distribution of all prenatal and postnatal Turner syndrome karyotypes (even though some of the prenatal diagnosed cases of Turner syndrome came to term, they are not included in the postnatal group). Results are given as number (%).

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Prenatal</th>
<th>Postnatal</th>
</tr>
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<tbody>
<tr>
<td>45,X</td>
<td>134 (64%)</td>
<td>162 (47%)</td>
</tr>
<tr>
<td>45,X/46,XX</td>
<td>45 (22%)</td>
<td>59 (17%)</td>
</tr>
<tr>
<td>45,X/46,X,i(Xq)</td>
<td>9 (4%)</td>
<td>41 (12%)</td>
</tr>
<tr>
<td>45,X/46,X,i(Xq)/47,X,i(Xq)</td>
<td>15 (7%)</td>
<td>27 (8%)</td>
</tr>
<tr>
<td>45,X/46,X,i(Xq)/47,X,i(Xq)</td>
<td>15 (7%)</td>
<td>27 (8%)</td>
</tr>
<tr>
<td>45,X/46,X,i(Xq)</td>
<td>5 (2%)</td>
<td>16 (5%)</td>
</tr>
<tr>
<td>45,X/46,XX</td>
<td>5 (2%)</td>
<td>16 (5%)</td>
</tr>
<tr>
<td>45,X/46,XX/47,XXX; 45,X/47,XXX; 45,X/47,XX/48,XXX</td>
<td>5 (2%)</td>
<td>16 (5%)</td>
</tr>
<tr>
<td>45,X/46,X,r(X)</td>
<td>20 (6%)</td>
<td></td>
</tr>
<tr>
<td>45,X/46,XY</td>
<td>10 (3%)</td>
<td></td>
</tr>
<tr>
<td>Others with Y material</td>
<td>11 (3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>209 (100%)</td>
<td>346 (100%)</td>
</tr>
</tbody>
</table>

*i(Xq) = isochromosome X(q).*
the median age at diagnosis was 15 years, with a range of 0–86 years (C H Gravholt, K Stockholm and S Juul, unpublished observations) (Fig. 2).

Morbidity and mortality

Morbidity is clearly increased in Turner syndrome. In a study of all diagnosed females with Turner syndrome (n = 594; years at risk = 54,10 years) and the background population of women (n = 2,594,036) in Denmark, we compared incidence rates of diseases suspected to occur with increased frequency (8). The relative risk (RR) of an endocrine diagnosis in Turner syndrome patients is increased to 4.9 (95% confidence interval (CI) 3.6–6.4), being accounted for by an increased risk of hypothyroidism (RR: 5.8; 95% CI: 1.2–16.9), thyroiditis (RR: 16.6; 95% CI: 3.4–48.5), type 1 diabetes (RR: 11.6; 95% CI: 5.3–22.0), and type 2 diabetes (RR: 4.4; 95% CI: 2.4–7.7). Likewise, the risk of ischemic heart disease and arteriosclerosis (RR: 2.1; 95% CI: 1.2–3.3), hypertension (RR: 2.9; 95% CI: 1.2–6.0), and vascular disease of the brain (RR: 2.7; 95% CI: 1.04–5.3), was increased. The risk of other conditions like cirrhosis of the liver (RR: 5.7; 95% CI: 1.6–14.6), osteoporosis (RR: 10.1; 95% CI: 2.2–30.9), and fractures (RR: 2.16; 95% CI: 1.50–3.00) was also increased, as were the risks for congenital malformations of the heart, of the urinary system, of the face, ears, and neck. The relative risk for all cancers was 1.35 (95% CI: 0.70–2.35), with only the risk of colon and rectal cancers being significantly elevated (RR: 4.94; 95% CI: 1.02–14.45). Congenital malformations were most frequent among women with the 45,X karyotype, while endocrine diseases, heart disease, hypertension, and arteriosclerosis were more frequent in women with other Turner karyotypes (8). Two studies in Denmark using two different and independent registries, namely the Cancer Registry and the Danish National Registry of Patients, found the overall risk of cancer to be comparable to the background population (8, 29). Only the risk of cancer of the colon was uniformly found to be increased, possibly due to estrogen deficiency, since postmenopausal women do seem to have a higher risk of this cancer, while hormone replacement therapy (HRT) in this setting reduces this risk (30). Mortality is also increased in Turner syndrome. In a British cohort study (n = 400, years at risk = 8609, deaths = 62) the relative risk of death was increased to 4.2 (95% CI: 3.2–5.4) (31), with increases due to diseases in the nervous, digestive, cardiovascular, respiratory and genito-urinary systems. Death due to cancer was lower than expected, corroborating morbidity studies from Denmark, where cancer incidence has also been found to be low, except the risk of cancer of the colon (8, 29). Earlier, Price et al. also found mortality to be increased threefold, especially in females with congenital malformations (32). Furthermore, they found aortic dissection to be the cause of death in three cases, which was greatly in excess of the expected level. Omitting patients with congenital malformations from the statistical analysis reduced the mortality ratios to normal levels.

Conclusion

The current knowledge of genetics in Turner syndrome does not explain a significant proportion of the phenotypical characteristics of the syndrome. The diagnosis ‘Turner syndrome’ is easy in typical cases, but is often difficult, leading to a quite remarkable delay in
diagnosis, as well as non-diagnosis. It is also clear that a number of females with Turner syndrome are never diagnosed. Whether these ‘non-diagnosed Turner syndrome females’ are different phenotypically is obviously not known. Morbidity and mortality are increased, but the etiology of the abnormalities that leads to this rather substantial increase is not clear.

The pituitary–ovarian axis

Morphology

In the 1960s Singh and Carr, and Carr et al. studying ovaries from fetuses with TS with the 45,X complement, showed that the germ cell count seemed normal until week 18 of gestation, after which accelerated degeneration occurred (33, 34). Speed suggested that the infertility associated with TS was due to this loss of germ cells, and that it was initiated by the inability of the lone X chromosome to pair with an autosome during meiosis (35). The gonadal insufficiency is associated with high levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in early childhood (2–5 years) and after the time of normal onset of puberty (11 years) (36), while during the neonatal period and late childhood the levels of FSH and LH are comparable to those in healthy girls (36, 37). The gonadotropin pulse periodicity of FSH and LH is normal in the prepubertal years (38). In adulthood, as in other conditions of hypergonadotropic hypogonadism, the levels of FSH and LH are increased to menopausal levels. The view that apoptosis of germ cells in TS is almost complete in the first years of life has recently been challenged by two studies. Hreinsson et al. obtained ovarian biopsies from nine females with Turner syndrome (12–19 years; 4 had pure 45,X), and found 1.5–128 follicles per mm³ of the ovarian cortical tissue (39). The authors concluded that cryopreservation for later treatment of infertility might be an option in Turner syndrome. We found that inhibin A and/or B were undetectable in most girls without signs of ovarian function (menstruations and pubertal development). However, a few girls did have detectable levels of inhibin A and/or B (40). During the normal menstrual cycle, inhibin B concentrations increase in the early follicular phase in response to the rising FSH concentration exerting negative feedback on the pituitary (41, 42), and it is primarily produced by the granulosa cells of the small developing follicles (43). The inhibin A concentration increases prior to ovulation, increases further after ovulation, and reaches peak values in the mid-luteal phase, primarily being produced by the granulosa cells of the dominant follicle and the corpus luteum (41, 44). The data from the studies by Hreinsson et al. and Gravholt et al. suggest that there may indeed be viable follicles present even in classical Turner syndrome (39, 40), and this may explain why 30% or more of females with Turner syndrome show signs of puberty (45), suggesting residual ovarian sex hormone production. Thus, a thorough evaluation of reproductive capacity is relevant in pubertal females and young adolescents with Turner syndrome. Another recent study, however, utilizing terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) in the ovaries of 4 Turner syndrome fetuses (15–20 weeks of gestation) found massive apoptosis of the oocytes (50–70% of cells, in comparison with 3–7% of oocytes in normal fetuses) (46). Reynaud et al. recently examined 10 aborted fetuses with 45,X, and found follicle formation and growth severely reduced (47). These latter studies support early studies of increased and rapid apoptosis taking place already in utero. Hypothetically, it could be speculated that early estradiol substitution might rescue oocytes from apoptosis. A search for growth factors and other compounds participating in ovarian maintenance is underway.

Function of the pituitary–ovarian axis

The untreated female with Turner syndrome

The average age of menarche in girls varies, by country, by social environment, and by ethnicity. For many years most authors advocated postponing the age of inducing puberty as long as possible in order to achieve an optimal growth response to growth hormone (GH) treatment. However, the most ideal timing of endocrine therapy allows induction of puberty in conjunction with the patient’s peers to avoid social problems at school because of delayed physical and psychological development. This would probably also allow optimal bone mineralization to take place (see below). In most normal girls puberty starts around the age of 12 years. However, since up to 30% of girls with TS will undergo some spontaneous pubertal development and 2–5% will have spontaneous menses and may have the potential to achieve pregnancy without medical intervention (45, 48, 49), signs of puberty should be looked for before starting estrogen therapy. When FSH and LH are clearly elevated, and clinical signs of puberty are missing, pubertal induction should be started. It can be postponed for a while, if the individual patient does not suffer from psychological distress because of lack of signs of puberty, when comparing herself to her peers. Pubertal development may be delayed if it occurs at all, and, in most cases, is followed by progressive premature ovarian failure.

Effects of hormone replacement therapy

In order to induce pubertal development, the dosing and timing of estrogen therapy should probably aim at mimicking normal pubertal development, taking into account the individual’s desire to begin puberty. Doses should be individualized starting with very low doses of estrogen as monotherapy, which may be monitored in terms of the development of secondary sex characteristics,
serum LH and FSH, bone maturation or uterine volume. A gestagen is added when breakthrough bleeding occurs. Estrogen therapy should be coordinated with the use of GH. This should be individualized for each patient, so as to optimize both growth and pubertal development. When growth is a priority, delaying estrogen therapy may be an option to avoid compromising adult height (50). A recent growth promoting trial has documented that a physiological timing of estrogen therapy does not compromise adult height, when GH therapy is started early and the dose is increased stepwise (51). A retrospective study showed a growth-stunting effect of appropriate timing of pubertal induction (50), and suggested that GH has a growth-promoting effect only in the first 2 years after institution of estradiol treatment, while another retrospective study showed no difference in adult height among GH-treated girls with spontaneous puberty (12.4 years) and girls with late induced puberty (14.5 years) (52). In addition, proper estrogen replacement during puberty has positive effects on motor speed, verbal and nonverbal memory and processing (53, 54), although proper HRT during adulthood does not seem to correct some of the neuro-cognitive deficits found in Turner syndrome (deficits in visual-spatial abilities, visual-perceptual abilities, motor function, nonverbal memory, executive function, and attention abilities) (55). However, it is not known whether these deficits are sensitive to the dosage of estrogen. Thus, future studies are needed in order to clarify unresolved issues, i.e. when to start pubertal induction, the stepwise increase in estradiol dose, which replacement dose to use once pubertal induction is finished, with respect to uterine development, bone mineral accrual, the cardiovascular system, and finally, if any route of delivery (i.e. oral, transdermal (gel or patches), or injections) is superior to others.

Fertility

Since most women with Turner syndrome have ovarian dysgenesis, and thus are infertile, this aspect has also to be dealt with. Adult women have rated infertility as their most prominent problem of the syndrome (56), and most, but not all, recent studies have shown promising results with oocyte donation (49, 57–59), which is an option in many countries. The most recent studies have shown better results than earlier studies, and comparable with oocyte donation in other groups of patients. One factor may be better preparation of the uterus for implantation (uterine size and endometrial thickness) with prolonged treatment with high doses of estradiol (4–6–(8) mg of 17β-estradiol) (58). A future scenario could be to offer the possibility of cryopreserving ovarian tissue from young girls/adolescents with Turner syndrome taken as laparoscopic biopsies as a part of an in vitro fertilization program. Such a program would have to be in cooperation with the pediatric department in order to extend the offer before final degeneration of follicles. Technically, this should be possible (49) although ethical considerations may intervene.

Conclusion

A range of issues related to the function of the pituitary–ovarian axis remain unsolved. This includes the appropriate age at pubertal induction, and the appropriate HRT dose once regular menses have been achieved, aiming at creating maximal peak bone mass and an adult sized uterus. More research is needed to examine the status of the ovarian failure in Turner syndrome – when is the ovarian demise complete, what endocrine, paracrine, immunological factors/factors are missing, can ‘ovarian rescue’ be performed, etc? How should the issue of infertility be dealt with in the future? As the in vitro fertilization techniques improve the prospects for women with Turner syndrome may improve alongside. Ovarian implantation after cryopreservation may be an option. The recent observation of intrauterine apoptosis shows the importance of operative molecular mechanisms during critical stages in embryogenesis (46). With the current state of knowledge puberty should be induced at an appropriate age in comparison with peers. Estradiol should preferably be used in slowly increasing doses until bleeding occurs, when a gestagen should be added and cyclical treatment commenced. HRT should be continued until the age of natural menopause. Only regimens with continuous estrogen exposure should be used, since TS women become absolutely estrogen depleted if regimens with a ‘pill-free week’ are used. Bone status and secondary sex characteristics should be monitored.

The growth hormone–insulin-like growth factor (GH–IGF-I) axis

Spontaneous growth in Turner syndrome

Since short stature is almost always present in Turner syndrome, with adult height approximately 20 cm shorter than controls of the same ethnicity (60–68), much emphasis has been put on the GH–IGF axis. Growth in Turner syndrome is mildly retarded already in utero (60, 69, 70) and is also subnormal during early childhood (69–71). In addition, there is a delayed onset of the childhood component of growth and a slow growth during childhood. The normal pubertal growth spurt is virtually absent in Turner syndrome (62), even in girls with spontaneous puberty. The genetic basis for the growth stunting in TS still needs to be unravelled. The recent cloning of a novel gene from the pseudoautosomal region (PAR1) on the X and Y chromosome, named SHOX (72) or PHOG (73) and subsequent studies of expression point towards the
involvement of the gene in longitudinal growth and bone development during normal childhood. Haploinsufficiency of the SHOX gene (normally two copies of this gene are expressed and haploinsufficiency will lead to a decrease of function (possibly in a dose-dependent manner)) is seen in TS and in Leri-Weill dyschondrosteosis, another syndrome showing some of the abnormalities seen in TS. SHOX is exclusively expressed in the developing limbs and in the first and second pharyngeal arches and may thus contribute to the understanding of the mesomelic short stature and other skeletal features of TS, i.e. short fourth metacarpal bones, cubitus valgus, high arched palate, micrognathia and Madelung deformity. It is suggested that SHOX is a repressor of growth plate fusion and skeletal maturation in the distal part of the limbs (74), implying that haploinsufficiency of SHOX could lead to the premature growth plate fusion in these (75). This may also explain the distinct dysproportionality of the skeleton (see below) (76). Furthermore, Kosho et al. suggested that gonadal estrogens exert a maturational effect on distal skeletal tissue susceptible to premature growth plate fusion because of the SHOX haploinsufficiency, explaining why the skeletal features may not be apparent before spontaneous puberty or induced puberty (77). Haploinsufficiency of SHOX, however, cannot explain all growth reduction in TS, and the verdict is still open as to what causes the remainder of the growth reduction (77). Also, premature skeletal fusion is not a concern in TS where bone age is normally retarded. Neither has it been clarified why adult height, and thus a normal growth pattern, can be normalized during GH therapy in TS. It is, however, a fact that adult height may normalize when patients are given large and escalating doses of GH (78, 79). The rationale for treating girls with Turner syndrome is not merely a lack of GH secretion, but probably rather the poor growth due to SHOX haploinsufficiency and possibly other genetic effects.

**GH—IGF activity**

**GH secretion in Turner syndrome** Recent studies in girls younger than 9 years, as well as in older girls, are equivocal and the spontaneous and/or stimulated GH secretion has been found to be diminished by some (80–84), while others have found a normal GH secretion (85–88). In adults, the spontaneous 24-h secretion of GH is reduced by 50% in comparison with age-matched controls (89); however, this reduction in GH secretion can be explained by differences in body composition. The bioactivity of circulating GH has been reported to be reduced (90) and various GH isoforms have been found to prevail by some (91), but not by others (92). Urinary 24-h GH excretion has also been found to be in the range of that of GH-deficient children (93), and to normalize in response to GH treatment. The normal increase in GH secretion during puberty is absent in Turner girls (80), but can be partially restored by replacement of sex hormones (80, 84), without, however, restoration of the abnormal growth rate. Girls with Turner syndrome have fewer GH pulses during the night (94), and adult women with Turner syndrome secrete GH with increased irregularity (disorderliness) when assessing 24-h sampled series (95). Normal women secrete GH with greater irregularity than men and this has been interpreted to arise from a more complex network coordinating GH release (96). Pincus et al. found that 5 weeks of low-dose estrogen treatment significantly increased irregularity in girls with previously untreated Turner syndrome (96), while in adult TS women, HRT does not affect the irregularity of GH secretion (95). A relative lack of androgens exists in post-pubertal Turner girls (97, 98), which may participate in the higher irregularity of GH secretion in Turner syndrome. GH binding protein (GHBP), the extracellular part of the GH receptor, is present in the circulation and binds GH. GHBP exists in molar excess of basal GH and the amount of GHBP increases with increased circulating GHBP both in children (99) and in adults (89), although in one study GHBP was comparable to controls (100). GHBP is not increased by GH treatment (99), while the effect of pubertal induction with estrogen has been found to increase GHBP in one study (101), but was without effect in another (102). The level of GHBP has been linked to the degree of adiposity (103), and in one study the higher level of GHBP in Turner syndrome was associated with differences in body composition (89).

**IGF-I bioactivity** IGF-I is the effector hormone of some of the growth actions of GH (104); in girls with Turner syndrome levels of serum total (extractable) IGF-I are normal between ages 4 and 9 years, but the pubertal rise is absent and IGF-I is therefore lower between ages 11 and 16 years of age in comparison with normal age-matched girls (105). Others found IGF-I to be uniformly reduced during childhood (80), or comparable to age-matched controls (84). Low-dose estrogen therapy has been shown to increase IGF-I (105, 106), while large doses of estrogen suppress circulating levels of IGF-I in normal individuals (107). Untreated adult patients with Turner syndrome have serum levels of total IGF-I and II not statistically significantly lower than controls, but they do have lower levels of free IGF-I (108). However, in a larger setting (n = 60) of adult women we recently found that total IGF-I was also significantly diminished (76). Serum levels of IGF binding protein (IGFBP) -1, -2, and -3 are within the normal range (89). Estrogen therapy increases IGFBP-1 (109, 110), which will tend to lower free IGF-I (111); IGFBP-1 in itself is considered to be an inhibitory IGF binding protein (112). In accordance with these data, we found diminished levels of free IGF-I which is probably an important bioactive fraction.
of circulating IGF-I. In addition, markedly increased proteolysis of IGFBP-3 was found (108). Increased IGFBP-3 proteolysis could imply reduced IGF-I binding capacity and thus increased clearance of IGF-I. IGFBP-3 is the major carrier protein for circulating IGF-I (and IGF-III), and has, in addition, independent actions of its own, since fragments of IGFBP-3 inhibit growth of cells (113). Thus, although the general belief was that the GH–IGF-I axis is normal or near normal in Turner syndrome, these recent results point at a partially disturbed GH–IGF–IGFBP axis, with low free IGF-I and high circulating levels of IGFBP-3 fragments. The concept of partial GH and/or IGF resistance was proposed long ago (114). This resistance has not, so far, been studied in great detail. However, the fact that girls with Turner syndrome need larger doses of GH for several years than GH deficient patients and others, to reach predicted height (see below) indirectly proves the existence of GH/IGF resistance. Whether this is related to GH, GH binding to circulating GHBP, the GH receptor, intracellular generation of IGF-I, or is related to IGF-I, the IGF-I receptor, or intracellular actions of IGF-I is not known.

**Growth promoting treatment in Turner syndrome – growth hormone and other agents**

**Effects upon height and the skeletal system** Initial short-term studies using GH showed pronounced acceleration of height velocity during short-term treatment (115–120). However, an increasing number of studies found less promising results in terms of adult height (121–131). In some trials GH treatment has been combined with oxandrolone with an additive height increment (132–138). A few studies found better results with GH treatment (79, 137). Recently, one study showed normalization of adult height, using large doses of GH (51, 78). In this study 68 girls were randomly assigned to three treatment groups. All groups received high dose GH treatment for 7 years: group A, 4 IU/m²/day; group B, 4 IU/m²/day for 1 year, then 6 IU/m²/day; group C, 4 IU/m²/day for 1 year, 6 IU/m²/day in the 2nd year, then 8 IU/m²/day. The girls received GH at 6.5 years (2–11 years) of age. Estrogen therapy was started at 12 years of age in most girls. Adult height data are available and they indicate that a normalization of height was attainable, in comparison with healthy Dutch girls (51). The response was dose-dependent and most girls reached a height above 150 cm. The study pinpointed four aspects of the problems seen in previous studies. (i) An early start of GH treatment is important, (ii) escalating dosing regimes can overcome the waning effect of GH treatment after 1–2 years, (iii) starting pubertal induction with estrogen at an age appropriate in comparison with the peers of the Turner girls does not compromise growth, and (iv) a normal adult height can be attained in Turner syndrome. Another recent study had similar results with an escalating GH dose regimen (79).

GH treatment seems to increase bone mineral density (BMD) in girls (139, 140), although in these two small studies no untreated control group of girls was included, and the patients were followed for up to 2 years only. However, during short-term (2 months–1 year) GH treatment a decrease in BMD takes place, although it increases collagen turnover (as an indirect marker of osteoclastic function), and osteoblastic function, as assessed by an increase in serum osteocalcin (141) and alkaline phosphatase (142), which would predict increases in BMD during longer term treatment. This initial decrease in BMD during GH treatment can be entirely explained by the concomitant increase in both bone resorption and bone remodelling, which results in negative bone balance, later to be replaced by a positive bone balance and an increase in BMD (after more than 2 years of treatment). In a 3-year treatment study of GH and estrogen, increases were recorded in BMD measured using phalangeal radiographic absorptiometry. The study group (n = 19) was followed for an additional 3 years after discontinuation of GH treatment (during continued estrogen treatment), and BMD increased further, being comparable to increases in normal girls (143). However, this study cannot discriminate between estrogenic effects on bone and any possible additive GH effect. In a small longitudinal study (n = 8), females with Turner syndrome, who previously had received GH treatment and subsequently estrogen, and initially had normal BMD levels, did obtain a decreased lumbar BMD (but normal femoral neck BMD) as young adolescents (144). Since areal BMD measurements are problematic (see below) in Turner syndrome, one study looked at volumetric lumbar BMD in a group of young adults (17–25 years; n = 26) and found no additive effect of GH treatment (to estrogen treatment) for 5 years in comparison with a matched control group of Turner syndrome females receiving only estrogens (145). High dose GH during long-term treatment (7 years) possibly increased phalangeal volumetric BMD (146). After 4 years of treatment, low dose estrogen treatment was added, and the volumetric BMD standard deviation score increased; subsequent analysis of variance suggested that the increase was attributable to GH, possibly in a dose-dependent manner. However, caution must be exercised, because (i) no control group was included, (ii) lumbar and femoral neck BMD was not assessed, and (iii) a pure effect of estrogen cannot be excluded. Furthermore, it must be remembered that the extremities are affected by the SHOX haploinsufficiency in Turner syndrome (mesomelia), whereas the lumbar spine is not, emphasizing that assessment of bone status in Turner syndrome must include measurement of BMD at the level of the spine, the femoral neck, the arm, as well as whole body BMD. Estrogen therapy increases height velocity in Turner syndrome during short-term treatment.
(106, 147), however, it does compromise long-term growth and can no longer be advocated as the sole treatment for the advancement of height (148). Still, a couple of studies have shown that combined treatment with oxandrolone and low dose estrogen therapy increases height moderately (3 cm) (149), or has no effect on adult height outcome (150). Oxandrolone treatment increases height velocity and adult height in most (151–153), but not all (61) studies.

Effects upon GH/IGF parameters and intermediary metabolism GH treatment increases circulating levels of GH, IGF-I, and IGFBP-3 in a dose- and time-dependent manner (101, 142, 154). Combination therapy with GH and oxandrolone or testosterone treatment further increases IGF-I, but not IGFBP-3 (155, 156). Sex hormone binding globulin (SHBG) and IGFBP-1 decrease during GH therapy (142, 157), and decrease even further after the addition of oxandrolone (157). IGFBP-1 was consistently found to be inversely related to insulin levels, as found also in other situations. GH treatment does not affect serum testosterone in TS (157). GH affects intermediary metabolism in a predictable manner during short-term administration (4h), with increases in free fatty acids (154), while this effect is no longer discernable during longer term treatment (2 month) (142). Treatment with GH induces transient alterations in thyroid status, with an initial decrease in thyroxine, with subsequent return to baseline levels, and an increase in the serum level of thyrogbulin (158). The same effect of GH is seen in GH-deficient patients (159). In order to induce a comparable positive nitrogen balance as seen in GH-deficient patients during GH treatment, higher doses of GH have to be used in Turner syndrome (160).

Effects on carbohydrate metabolism Carbohydrate metabolism has been studied in some detail in TS, especially concerning the insulin resistance due to GH therapy, now widely offered as a growth promoting agent to many girls and adolescents with TS. Insulin resistance is also seen in GH-deficient patients treated with GH, and in acromegals. Caprio et al. studied seven GH-treated TS adolescents and found both first and second phase insulin secretion to be increased during a hyperglycemic clamp (161). The increase in insulin secretion was present before and was exaggerated during GH treatment. Similar results were attained by Stoppoloni et al. in 4 TS girls (162). In a recent short-term GH intervention study, we found insulin sensitivity to be comparable to age- and Tanner-matched controls at baseline (142). Insulin sensitivity was assessed both during an oral glucose tolerance test (OGTT) by a composite whole-body insulin sensitivity index (ISIcomp) (163), and by the homeostasis model assessment (HOMA) index based on fasting glucose and insulin concentrations (164). The participants were well matched for weight and body mass index (BMI), although dual densitometry x-ray (DXA) scans showed significant differences in regional adiposity (142). Most studies have also found a relative impairment of glucose tolerance or found insulin resistance during GH treatment (154, 157, 165, 166), while others have not (167). However, most agree that GH treatment induces insulin resistance, an effect that is reversible with discontinuation of treatment (166). Combined treatment with GH and oxandrolone induces more pronounced insulin resistance with higher insulin levels than with GH alone (157, 167).

Effects on cardiac function In acromegaly left ventricular hypertrophy is seen in addition to increased morbidity and mortality due to cardiovascular causes (168). These features are related to the excessive GH secretion, and has aroused concern that high dose GH treatment in TS might affect left ventricular function. Cardiac function per se is often different in Turner syndrome, due to the high frequency of congenital malformation, most commonly bicuspid aortic valves and coarctation of the aorta, but also including a number of other left-sided malformations (for review see reference 169). However, a number of other vascular conditions are also frequently seen, i.e. hypertension, dissection of the aorta, myocardial infarction, and cerebrovascular insults (see section on The Heart in Turner Syndrome below) (169). Few studies have addressed the effect of GH treatment of TS on the heart. In the recent Dutch study with very high doses of GH for, on average, 7 years (78), no signs of left ventricular hypertropy or evidence of worsening of hypertension were found, except in two girls with pre-existing left-sided obstruction (congenital abnormal aortic and mitral valve respectively) who developed significant valve stenosis, and thus possibly left ventricular hypertropy, and were excluded from further statistical analysis. This could suggest that pre-existing valve abnormalities may induce development of left ventricular hypertropy. At inclusion, many Turner syndrome girls had increased systolic and diastolic blood pressure in comparison with a normal reference material, but at the end of the study there was a slight decrease in the age-adjusted diastolic blood pressure (170). In a recent cross-sectional study of girls with Turner syndrome receiving either no treatment (mostly young girls), GH, or GH in combination with estradiol and a progesterin, 17% had elevated blood pressure in comparison with age-matched reference data (171). Furthermore, by 24-h blood pressure evaluation, it was shown that 57% of all girls had a blunted nocturnal fall in blood pressure (‘non-dippers’) (171, 172). Radetti et al. compared TS girls after 4.9 years of GH treatment with an age-matched control group, and found slight increases in heart rate and systolic blood pressure, as well as minor differences during echocardiography – changes suggested to be due to increased heart rate and reduced peripheral resistance (173). It remains to be seen if
there are any very-long-term effects of GH treatment on the cardiovascular system. It is recommended that very-long-term follow-up is performed in previous and ongoing GH treatment trials.

**Effects on cognition, quality of life and economic considerations** Females with Turner syndrome present a particular neurocognitive profile with impaired performance on motor tasks (174), impaired visual-spatial ability, but normal verbal skills (55, 175–178). Ross et al. tested neurocognitive performance in a randomized trial, and found no effect of GH treatment on the tested parameters (179). Few studies have dealt with an issue like quality of life in TS during GH treatment. One GH treatment study assessed self-concept and psychosocial perception (180). It was found that after 18 months of treatment, females in the GH arm versus the untreated arm had better scores on global self-concept, appearance, intelligence and peer realtionship, and parents reported less hyperactivity in the treated girls. Deficiency of estradiol may explain these impairments in cognition. In placebo-controlled double-blind trials it has been shown that ethinyl estradiol (12.5–50 ng/kg/day) positively affects nonverbal processing speed and motor function in 10- to 12-year-old girls with Turner syndrome (53), and that ethinyl estradiol (25 ng/kg/day) improves verbal and non-verbal memory in 7- to 9-year-old girls (54). Use of replacement doses of estradiol at an even younger age has not been carried out but would, from a physiological point of view, make sense. Oxandrolone supplementation (2 years) to girls at the age of 10–14 years resulted in an improved working memory compared with placebo (181). Before the recent publication of the Dutch GH study (51) showing a much higher gain in adult height than in previous studies, it was estimated by Bryant et al., in a cost-effectiveness model of GH treatment in Turner syndrome, that the incremental cost of each centimeter in adult height gained after 5 years of GH treatment was in the order of £16 000–17 400 (182). The model did not include much data on quality of life aspects due to lack of data, and there were a number of uncertainties, i.e. a possible underestimation of the effect of GH, the fact that most trials reported starting GH treatment later than recommended, etc. It is likely that renewed modelling of the incremental cost of an added centimeter would give a different picture today.

**Conclusion**

While the stunting of growth is at least partially explained by the discovery of the SHOX gene and the haploinsufficiency of this gene in Turner syndrome (and Leri–Weill syndrome), we still do not know whether other genes are responsible for the remaining characteristics or whether perhaps chromosomal imbalance may be involved. The finding of the SHOX gene has broadened our understanding, but has only partly explained the growth deficit. Studies focussing on the cellular mechanisms of GH and IGF action in Turner syndrome should possibly help us better to understand the growth stunting. Serum levels of all known relevant growth factors are normal or low-normal, but despite this the existence of GH and/or IGF resistance is proposed. Height in Turner syndrome can be normalized with large escalating doses of GH in TS. Long-term studies are needed in order to assess possible adverse effects of high dose GH treatment. Before starting GH treatment it is prudent to perform an echocardiography in order to detect structural abnormalities (see section on Turner syndrome and the heart), as well as monitor glucose homeostasis (HbA1c and/or fasting glucose) during and after treatment. IGF-I can be used as a monitoring tool, and should be in the range of 2 standard deviation scores (SDS), or just above the normal range (for a practical guide to GH treatment in TS, see (4)).

**Carbohydrate metabolism and physical fitness**

**Insulin sensitivity**

Studies performed during childhood studies have presented conflicting results. Caprio et al. found insulin resistance in girls with TS (183). They studied a group of young girls \( (n = 8, \text{ age } 10 \pm 0.8 \text{ (s.e.) years}) \) naive to GH and sex steroids, and a group of adolescents \( (n = 5, \text{ age } 17.6 \pm 1.4 \text{ (s.e.) years}) \) who had received or did receive estrogen therapy. Using a euglycemic hyperinsulinemic insulin clamp, they found reduced insulin sensitivity in both groups in comparison with age-matched controls. They performed indirect calorimetry and showed an impairment of non-oxidative glucose disposal (183). Cicognani et al. found impaired glucose tolerance more frequently in girls with Turner syndrome compared with controls, most markedly in young girls (184). Other investigators documented impaired glucose tolerance in 15% of girls with Turner syndrome (154, 167). An early observation showed that many adults with TS developed type 2 diabetes or glucose intolerance (185). In adolescents and adults a large proportion of TS patients exhibits impaired glucose tolerance or overt type 2 diabetes during an OGTT (184–188). In adults, we found a normal insulin sensitivity utilizing the Minimal Model to test intravenous glucose tolerance test (IVGTT) data in 26 adults (age 20–50 years) (188), but a relative impairment in first phase insulin response. This is regarded as a hallmark of development of type 2 diabetes (35). Insufficient first phase insulin response causes a delay in suppression of hepatic glucose production (36) which may contribute to glucose intolerance. Glucose effectiveness was found to be normal, still 50% were glucose intolerant after OGTT, despite
comparable levels of fasting glucose and insulin suggesting a defect in glucose disposal in TS (188) in accordance with early studies in girls which suggested a muscular defect (183). It is not entirely clear why this discrepancy between Minimal Model data and OGTT data was present. The Minimal Model has been found to underestimate insulin sensitivity in some situations and the adequacy of the single-compartment description of glucose kinetics has been questioned (189), and it may be that the insulin sensitivity index derived from the Minimal Model was not able accurately to distinguish between TS and controls despite other indications of insulin resistance. In a recent cross-sectional study of 71 adult TS, fasting levels of glucose and insulin were also comparable to those of controls, leading the authors to conclude that metabolic risk factors (e.g., elevated fasting glucose) were not a concern in TS (190). In an epidemiological study of the entire (diagnosed) population of TS females, we found type 2 diabetes to be very frequent (RR: 4.4), as well as type 1 diabetes (RR: 11.6) (8). Thus, a state of normal fasting glucose and insulin levels, but compromised glucose metabolism during stimulation seems to be present, including elevated 2-h glucose, and thus presumably also postprandial hyperglycemia. Both in pre-diabetic and non-diabetic populations the 2-h post-load plasma glucose level is a strong and independent predictor of increased cardiovascular mortality (191, 192). It is evident that impaired glucose tolerance, as well as diminished insulin sensitivity, is prevalent in TS. In one study in which muscle biopsies were taken, women with Turner syndrome (33±9 years) had an increased size of type IIa fibers, while the size of type I and IIx fibers were comparable to that of the control group. The groups did not differ in percentage of type I, IIa or IIx fibers and there was no difference in the capillary density. Significant correlations were found between insulin sensitivity and fasting insulin, and mean area of type IIa fibers. In addition, the capillary density correlated with insulin sensitivity and fasting insulin. It was concluded that otherwise healthy women with TS are characterized by impaired glucose tolerance, insulin resistance, low physical capacity, as well as enlarged type IIa muscle fibers indicating diminished oxygen and substrate supply for metabolic processes, which could be indicative of a prediabetic state (193).

**HRT and insulin sensitivity** Most females with TS are treated with HRT after induction of puberty. The effect of HRT on carbohydrate metabolism has only been looked at in one study. We found small changes in response to HRT using genuine 17β-estradiol and norethisterone. Fructosamine, as a measure of the average level of glucose during the preceding 14 days, and fasting plasma insulin levels were reduced significantly during HRT compared with no treatment, indicating improved glycemic control. Insulin sensitivity was unchanged (assessed using an IVGTT with Minimal Model analysis), while more study subjects had impaired glucose tolerance (OGTT) during treatment with HRT (188). Free fatty acid levels are higher in Turner patients despite a higher level of glycemia (188). This might suggest a defective suppression of the Randle cycle, perhaps mediated, in part, by inappropriately low levels of insulin (194). A positive family history of type 2 diabetes is found with unusually high frequency in several studies, but this does not fully explain the glucose intolerance, since Turner patients with a negative family history also exhibit these characteristics (185, 195–197). It is not clear what impact HRT has on insulin sensitivity and glucose tolerance in postmenopausal women. Intervention studies in postmenopausal women show that transdermal 17β-estradiol exerts either moderately positive effects on glucose metabolism (198) or no effect at all (199, 200). Oral administration of 17β-estradiol and norethisterone reduces the glycemic response after an OGTT in women with impaired glucose tolerance, without any change in the acute insulin response in some (201), but not in other (202) studies.

Other cross-sectional studies had diminished (203) or no effect of HRT on insulin sensitivity (204). In the latter study, the authors reported a close relationship between visceral adipose tissue and insulin sensitivity in women treated with HRT, with lower than expected insulin sensitivity at low amounts of visceral adipose tissue, but higher than expected insulin sensitivity at higher amounts of visceral adipose tissue (204). Most studies have reported a reduction of fasting glucose and insulin, which has been suggested to be due to increased clearance of insulin (205), and thus may not indicate improvement in insulin sensitivity. There is a serious problem in comparing the effect of HRT on insulin sensitivity in postmenopausal populations, given the inevitable age difference with a Turner syndrome population. Furthermore, most women with Turner syndrome suffer from extreme premature ovarian failure, with no or only a very short-term period of natural menstruations (45). Many females with Turner syndrome are naive to the effects of female sex hormones, although some of them experience natural or semi-natural puberty. In one study of young women with premature ovarian failure without TS, who could be compared with women with TS with respect to age, HRT has been shown to diminish insulin sensitivity (Minimal Model-derived insulin sensitivity (Si)) (206). Also Duncan et al. studied a population of relatively comparable women (surgically postmenopausal women, age 35–50 years, n = 22), but found no effect on insulin sensitivity (200). There are no very long-term controlled studies of the effect of HRT on glucose metabolism in patients with Turner syndrome or in otherwise healthy women of a comparable age. As regards cardiovascular risk factors, 3 major studies have recently been published and presented compelling data relevant to a postmenopausal population, but
again not easily applicable to a younger Turner syndrome population. In healthy postmenopausal women (aged 50–75 years; mean 63.3 ± 7.1 years) without prior cardiovascular disease the results from the recent Women’s Health Initiative randomized trial show that HRT should not be initiated for primary intervention against cardiovascular disease (30). In addition to an increased risk of cardiovascular disease in the treated group, the results showed increases in the risk of breast cancer, stroke, and pulmonary embolism, and a decreased risk of fractures and colorectal cancer in treated women (30). In postmenopausal women with a previous cardiovascular event (secondary intervention), HRT has proved to have no effect on cardiovascular mortality and morbidity (but also to have no detrimental effect) (207), and HRT has also been unsuccessful in postmenopausal women with a previous stroke, albeit not as deleterious as secondary intervention (208). Available data indicate that a large proportion of women with Turner syndrome have an abnormal glucose tolerance and inappropriate high levels of circulating insulin without and during replacement with sex hormones, which could indicate a deranged β-cell function (161, 162, 183, 185–188, 196). In animal models, glucose stimulated insulin release is reduced after ovariectomy and restored by replacement with sex hormones (209, 210). Thus, during longer term treatment with sex hormones an improvement in the indices of carbohydrate metabolism may take place, perhaps partly through the expedient effects of sex hormone replacement on physical fitness, body composition, and blood pressure (see below).

Physical fitness

Physical fitness (determined by sub-maximal VO$_2$$_{max}$ testing on a bicycle) of TS patients has been found to be diminished by 25% compared with control subjects (188), an effect which remains when differences in body composition are taken into consideration, and in this respect they are comparable to healthy relatives of patients with type 2 diabetes (211–213), along with reduced insulin sensitivity (214). In subjects with abdominal obesity and in healthy relatives to patients with type 2 diabetes it has been suggested that reduced physical fitness is associated with an increased number of type IIb muscle fibers (215). Furthermore, maximal oxygen uptake is dependent on the functional capacities of the lungs, cardiovascular system and muscle mitochondria (216). Interestingly, a recent study of aborted TS fetuses demonstrated the presence of heart and lung hypoplasia (217). If such a finding is reproduced in surviving TS subjects, this may explain part of the reduced physical fitness. Healthy women with Turner syndrome are characterized by impaired glucose tolerance and insulin resistance (see above), low physical capacity, and enlarged type IIa muscle fibers (see above) indicating diminished oxygen and substrate supply for metabolic processes. These abnormalities of muscle fiber composition and/or diminished capacity of the lungs and cardiovascular system might explain at least part of the diminished physical fitness of Turner patients. An increase in maximal oxygen uptake has been observed in patients with Turner syndrome during sex hormone treatment, concurrently with an increase in fat-free mass (FFM) (89). A progressive decline in muscle force is normally seen in postmenopausal women (218); however, this decline can be reduced by sex hormone replacement therapy (219). In a randomized trial of HRT with or without exercise and a control group (without exercise) it was shown that HRT with or without exercise was associated with increases in muscle performance, muscle mass, and muscle composition (220). The beneficial effect of HRT on muscle was greater when combined with exercise. In Turner syndrome patients, as well as in postmenopausal women, female sex hormones alone or perhaps via an increase in endogenous GH secretion seem pivotal in preventing a deterioration in both FFM and maximal oxygen uptake (89).

Conclusion

Although available evidence documents a number of defects known to lead towards overt type 2 diabetes, long-term studies are needed to examine possible positive effects of HRT (preferably with genuine 17β-estradiol and a gestagen) on the increased prevalence of abnormal glucose tolerance and type 2 diabetes. In addition, observational studies are needed to study the natural history of the development of type 2 diabetes in Turner syndrome. Treatment with GH induces insulin resistance in TS, as in other conditions, which subsides when treatment is discontinued. HRT induces small changes in glucose homeostasis, and it remains to be seen whether there are any long-term effects on the development of type 2 diabetes. Present recommendations are to continue proper HRT until the age of natural menopause (50–55 years).

Anthropometry and body composition

Basal situation

The adult height of Turner patients is approximately 20 cm below that of the average female population. There is no relation between karyotype and height or any other anthropometrical measurements in most studies (60, 64, 89, 221, 222), although a few authors have found certain karyotypes to be associated with increased adult height (66). The anthropometrical composition of women with Turner syndrome is very distinct: Turner females are primarily growth retarded via the longitudinal axis, while the horizontal measurements are comparable to those of control females (89,
223, 224). This means that while height, sitting height, and arm-span are reduced by approximately 3–4 S.D. compared with a reference population, hands and feet are reduced in size to a lesser extent, while head circumference, biacromial and bi-iliacal diameter are comparable to those of healthy women (Fig. 3) (68). BMI, waist/hip ratio (W/H), and fat mass (FM) have been found to be higher in adult Turner patients compared with age-matched controls, and FFM is lower, implying a higher incidence of adiposity (89, 187). In addition, we found distinct differences in regional body composition in young TS girls (9–15 years) in comparison with age- and BMI-matched controls. Total FM was increased in TS. This was accounted for by an increased FM in the arms and trunk. Likewise, FFM was decreased in Turner girls, although not reaching statistical significance (P = 0.09). Especially, FFM in the legs was decreased (significantly), while FFM in other regions was comparable to that of controls. Overall, bone mineral content (BMC) was diminished, being accounted for by reductions in BMC both in the legs and in the arms (142). In other words, Turner syndrome is a syndrome of disproportionate anthropometry and body composition.

**Effects of GH treatment**

Treatment with GH has seemingly beneficial effects on body composition with increases in muscle mass and decreasing amounts of body fat, as seen during 1 year of treatment and as studied by magnetic resonance imaging (MRI) of the thigh in girls with Turner syndrome (225). Likewise, we found that the FFM increased as determined by DEXA scans during short-term treatment (2 months) with GH. Treatment with GH reduced total fat mass in TS, especially in the arms and legs, and increased total FFM, primarily in the trunk (142). Similar changes induced by GH are seen in GH-deficient children and adults (226, 227). The 7-year Dutch GH study found a significant increase in the size of the feet, when controlling for the significant increase in height. None of the other body proportions (hand, head circumference, bi-iliacal and biacromial diameter, sitting height) changed significantly with GH treatment (228). Interestingly, discontinuation of GH treatment in the aforementioned study led to a slight increase in BMI 6 months later (229). At present, no GH treatment studies in an adult Turner syndrome population have examined a possible impact on body composition.

**Effects of HRT**

The menopause in healthy women is associated with a change in body composition towards a more android fat distribution and an increase in BMI (230), a situation which can be postponed by sex steroid replacement (231–236). In adult Turner patients a rise in FFM is recorded during sex steroid treatment without any change in BMI or W/H ratio. Turner women are characterized not only by increased amounts of body fat, but also by a more android body composition, and treatment with sex hormones slightly improves these abnormalities (188). There are no studies of the effect of discontinuing HRT. This remains interesting in the light of the recent studies of HRT in postmenopausal women.

**Conclusion**

Both anthropometry and body composition are abnormal in Turner syndrome. GH and HRT exert seemingly positive effects on body composition in Turner syndrome, and discontinuation of both GH and HRT is associated with detrimental effects. Long-term changes in body composition during both GH treatment and HRT have not been studied. Further, the dosage of HRT and thus a possible impact on body composition has not been studied.
Bone mineralization

Spontaneous accretion of bone mass

Peak bone mass depends on a number of factors, such as genetic background, nutrition, physical activity, local growth factors, and a number of hormones. In particular, a normal secretion of estradiol during puberty is required for normal skeletal mineralization (237–240). Recently, prepubertal estradiol secretion has been highlighted as essential for early accretion of bone, as evidenced in a study of a girl with aromatase deficiency and extremely low levels of estradiol, and, in addition, low bone density, responding to treatment with exogenous estradiol (241). It is evident that girls, and younger and middle-aged women with Turner syndrome have low areal bone density, as documented in numerous studies (242–249). There are, however, inherent problems in all these studies – namely the fact that TS subjects are small, and size is a major factor influencing areal BMD (and bone mineral content, BMC) measurements as determined by DEXA, because of the two-dimensional nature of DEXA scanning (250). Since reduced height is the almost universal finding in TS, addressing this issue is pivotal. Therefore, it is not possible to study BMD with the use of DEXA scans without acknowledging the influence of size. A recent study of adolescents calculated volumetric BMD of the spine and found values that were comparable to those of a reference population (145). In an adult population of 60 TS patients, we found slight, but significant reductions in volumetric BMD (vBMD) in the spine, while vBMD at the hip was actually greater in TS compared with an age-matched control group (76). However, areal BMD in the arm was severely reduced, pointing to a specific effect of the SHOX gene at this anatomic location, keeping in mind that the SHOX gene is specifically expressed in Turner syndrome (76).

Vitamin D metabolism was found to be abnormal, with a blunted response of serum 1,25-dihydroxy-vitamin D (1,25-(OH)2-D) levels to a low calcium diet (251), while calcitomin metabolism seems to be normal (252). However, two recent studies found normal or low levels of 1,25-(OH)2-D, but reduced levels of 25-hydroxy-vitamin D (25-OH-D), with increased levels of parathyroid hormone (PTH), suggesting normal conversion of 25-OH-D to 1,25-(OH)2-D, but either diminished intake (i.e. orally or from sunlight) of 25-OH-D or reduced uptake of the compound (76, 249). It is uncertain whether the reduced BMD is solely attributable to estrogen deficiency (243, 244, 246, 253). Recently, we observed that the increased risk of fractures in TS was present already in childhood and persisted throughout all age groups (8). This favours the view that the low BMD seen in TS is the result of both estrogen deficiency and other yet unknown mechanisms caused by the chromosome abnormalities or other (endogenous and exogenous?) factors. Often, adolescents with TS are introduced late to estrogens in order to avoid the stunting in growth conferred by the compounds, probably delaying and possibly reducing appropriate bone mineral acquisition. In one study we observed an association between age at start of HRT and vBMD in women with TS, suggesting that induction of puberty at an appropriate (and early) age is important (76). However, it is necessary to stress that the cross-sectional nature of this study makes it difficult to infer definite conclusions. In addition, minute amounts of circulating estradiol in normal prepubertal girls play a role in normal bone mineral accretion, and this aspect of prepubertal estradiol needs to be assessed in Turner syndrome.

Fracture risk

A few studies have looked at fracture frequency – one study in adolescents found an increased frequency of wrist fractures (254), while others did not (255, 256). A register-based study has suggested that osteoporosis (RR: 10.12; 95% CI: 2.18–3.20), and fractures (RR: 2.16; 95% CI: 1.50–3.00) are frequent diagnoses in a Turner population (8). The relative risk of fracture was increased for every location investigated (Fig. 4), but did not reach statistical significance for all locations, a result that could be due to few fractures at specific sites, because of too small a study population. This indicates that the decreased BMD seen in other studies leads to clinical consequences. The risk of fracture was already increased in childhood (Fig. 5). In order to further study the number and the sites of fractures, we conducted a questionnaire survey of all registered girls and women with Turner syndrome in Denmark. The fracture risk was found to be significantly increased by 25% in females with TS. This is less than found previously (100% increase (8)). However, it fits well with BMD measurements from clinical studies. Using our own previously published BMD...
values (76), we calculated the expected fracture risk from the data of Marshall et al., and found a relative risk of fracture of 1.39 and 1.42 at the spine and hip respectively in TS (257). Furthermore, the increased risk was largely restricted to forearm fractures. In addition, the age at which a fracture occurred was significantly reduced.

HRT, GH, and bone

Sex hormone replacement therapy is considered crucial to avoid a rapid decrease in BMC (253). Treatment with estrogens is needed to induce maximal peak bone mass in adolescents and young adults (145, 253, 258, 259). This is supported by four longitudinal studies of estrogen deficient as well as estrogen replete adolescents with TS. In these studies patients with spontaneous menstruations had normal BMD, while patients without menstruations had reduced BMD (260, 261). Further, GH seems to improve BMD (139, 140), although in these two small studies no untreated control group of girls with Turner syndrome was included, and the patients were followed for up to 2 years only. In a recent 7-year study with GH treatment given at three different doses (see above), where bone mineral density was studied by phalangeal radiographic absorptiometry, volumetric bone density was found to be normal or increased in a dose-dependent manner (146). However, estrogen was added after 4 years of previous GH treatment, and it is difficult to ascertain the individual effects of GH and estrogen in this study. Most (83%) adult Danish women with TS do receive HRT (262). Recently, a 3-year longitudinal study of 21 women with TS (aged 20–40 years), with iliac crest biopsies before and 3 years after treatment with HRT showed compelling effects of estrogen on bone. The women were treated with estradiol implants (and oral gestagen cyclically) (263). The implants resulted in estradiol levels comparable to levels normally present in premenopausal women, and considerably higher than the levels achieved with the regimens (estradiol 2 mg orally or equivalent transdermal doses, i.e. traditional HRT regimens used for postmenopausal women) used hitherto. Bone biopsies showed an increase in cancellous bone volume, a decrease in activation frequency, but the active formation period was increased. Wall thickness was increased, indicating an increase in bone formed at each individual remodelling unit. In parallel, BMD at the lumbar spine and femoral neck increased by 13% and 8% respectively. The data were interpreted as evidence of an anabolic effect on the skeleton of estradiol at these doses in young women with Turner syndrome (263).

Conclusion

Untreated girls and women with TS have low bone mass, but this seems to be quite easily overcome by adequate (both in timing and dosage) and appropriate GH and estradiol treatment. No very-long-term studies (both follow-up and intervention studies) of the effect of estradiol have been published. There is a definite need for such studies to determine the ideal treatment regimen during adolescence for achieving two goals: (i) attaining maximal peak bone mass and maintaining BMD without compromising adult height, and (ii) with appropriate timing of pubertal induction to achieve appropriate secondary sex characteristics. Furthermore, the dosage of estrogen during adult life has to be determined, which has been highlighted by the recent paper by Khastgir et al. (263). There are indications that the present dosage (i.e. 2 mg estradiol or equivalent) is insufficient in terms of bone health and also with reference to secondary characteristics. However, it is at present unknown how a higher dose may influence other aspects of adult TS. In addition, the role of selective estrogen receptor modulators (SERMs), such as raloxifin, in the treatment of osteoporosis in TS has to be determined.

Thyroid and adrenal function

Thyroid function

Thyroid dysfunction is common in TS. Hypothyroidism is frequent, and thyroid antibody formation even more so, especially in a subgroup with an isochromosome of the long arm of the X chromosome (i(Xq)) (260, 264–275), and as many as 30% or more eventually develop hypothyroidism. In a recent study, in which none of the participants had clinical hypo- or hyperthyroidism (as this was an exclusion criterion), levels of thyrotropin (TSH) were higher in Turner patients than in controls (276). Thus, adult Turner subjects seem to suffer from compensated hypothyroidism, which often progresses into overt hypothyroidism. Compensated hypothyroidism and insufficiently treated hypothyroidism are suggested to be associated with coronary artery disease, elevated low density lipoprotein cholesterol, and apolipoprotein B levels (277–279),
which may explain part of the increased risk of cardiovascular disease in Turner syndrome (8). However, it remains an enigma why so many TS females have thyroid autoimmune disease. The basis for the grossly increased risk of autoimmunity in Turner syndrome (also including celiac disease (280), and diabetes (see above)) is unaccounted for, and a genetic basis seems probable. A recent study shows increased tumor necrosis factor receptor- and CD95-mediated apoptosis in cord blood T-cells (CD4 + and CD8 + cells) (281), and earlier studies found minor deficiencies of humoral and cellular immunity (114, 245, 282–286), and it may be that these more or less discrete deficiencies in combination explain the increased risk of autoimmunity. GH treatment does not increase the frequency of auto-antibodies, although the percentage of antithyroid auto-antibodies did increase during treatment; however, no control group was included, and the number of patients with antithyroid antibodies does increase with age (287).

Whole-body protein metabolism has been found to be normal, and unaffected by the administration of estrogens to prepubertal Turner girls (288). Similarly, we found that energy expenditure was unchanged by treatment with sex hormones and not different from controls (89).

### Adrenal function

Adrenal function is normal in Turner syndrome, when tested with synthetic adrenocorticotropin (ACTH) (289), and not changed by concomitant treatment with estrogen (290), or GH (289), although one study found discrete changes during GH treatment, with increased responsiveness of adrenal steroidogenesis (the A5 pathway) to ACTH testing (291). In addition, a high frequency of unexplained heterozygosity for 21-hydroxylase deficiency has been found in Italian Turner TS (292). However, adrenarche does seem to occur earlier in girls with Turner syndrome without ovarian function, in comparison with controls and Turner syndrome girls with spontaneous menstruation, while pubarche is delayed in the former group (293).

### Conclusion

Hypothyroidism with antibody formation is extremely common in TS and has to be looked for continuously. Treatment consists of levothyroxine, and in some cases lithium, in the usual doses.

### Androgen insufficiency in Turner syndrome

Since approximately half of the testosterone production in normal females originates from the gonads, one would anticipate that Turner syndrome patients are also androgen deficient, which indeed has been reported (97, 98). It is clear from several studies that early introduction of estrogens can increase initial growth velocity, but has detrimental effects on adult height, due to accelerated maturation of bones and premature fusion of growth plates (294). Hence, with the androgen axis also being perturbed in Turner syndrome, both in adolescents and in adults, and since the interplay between the GH axis and androgens is documented, not least during the pubertal growth spurt, there may be a rationale for treating females with Turner syndrome with androgens. At present, this interplay has not been studied in great detail, although oxandrolone has been shown to increase adult height when used in conjunction with GH treatment (135, 295). However, the use of GH in combination with oxandrolone further increases levels of insulin and insulin resistance (157). No studies have addressed the issue of androgen replacement in adults. Androgen supplementation could be postulated to have beneficial effects on the excess of sexual problems, which has been described in Turner syndrome (56). In addition, androgen supplementation would possibly have positive effects on the reduced bone mineral content, manifest osteoporosis and an increased incidence of fractures (8, 242, 243), as well as the characteristic anthropometry and body composition of Turner syndrome (68). Most adult women with TS receive HRT which might further reduce circulating androgens (98), possibly by increasing levels of SHBG.

### Hepatic function

#### Spontaneous hepatic function

Few reports have evaluated hepatic function in Turner syndrome (296–298). One study found elevated levels of hepatic enzymes in 80% of middle-aged women with Turner syndrome, but could not associate the findings with overt hepatic disease (296). We found serum levels of alanine aminotransferase, γ-glutamyl transferase, and total alkaline phosphatase to be higher in Turner patients compared with controls. The elevated levels of some hepatic enzymes and proteins are not associated with overt hepatic disease, and it should be emphasized that women with Turner syndrome do not seem to consume more alcohol than other women (89). Still, recent epidemiological evidence suggests that cirrhosis of the liver is more frequent in Turner syndrome (8). In a recent study liver biopsies were performed in 27 women with Turner syndrome, because of persistently elevated liver tests (299). Multiple abnormalities were found, including marked nodular regenerative hyperplasia ($n = 6$), multiple focal nodular hyperplasia ($n = 2$), and cirrhosis ($n = 2$), associated, in some, with obliterator portal venopathy. Other patients showed more moderate changes, including portal fibrosis, inflammatory infiltrates, and non-alcoholic fatty liver disease. One patient underwent liver transplantation. The authors...
concluded that the main causes of liver abnormalities in Turner syndrome are vascular disorders thought to be congenital in origin, and non-alcoholic fatty liver disease, without signs of liver toxicity from concomitant estrogen therapy (299). The study is important, since it is the largest, includes liver biopsies, as well as thorough evaluation of other causes of liver disease, excluding viral, auto-immune and alcoholic causes, and because it apparently excludes estrogen therapy as a player in the liver abnormalities. Interestingly, a recent study of women with primary biliary cirrhosis (and not Turner syndrome) showed an increased frequency of X monosomy in peripheral white blood cells, as well as in subpopulations of white blood cells (300). The authors suggested that haploinsufficiency of specific X chromosome-linked genes leads to female susceptibility to develop primary biliary cirrhosis.

**HRT and liver function**

Rather unexpectedly we found a significant decrease in the level of liver enzymes after treatment with sex hormones, irrespective of route of administration (oral or transdermal 17β-estradiol) (89). This contrasts partly with previous comparisons of transdermal versus oral substitution therapy in postmenopausal women (301), in whom both treatment modalities caused an elevation of hepatic enzyme levels, and in Turner girls (n = 8) where low-dose (designed for pubertal induction) transdermal 17β-estradiol caused less of an increase in proteins produced in the liver, although liver enzymes were not assessed (302). Although sex hormone treatment in our hands had a positive effect on measures of hepatic function in adult Turner syndrome patients, several indices of hepatic function were persistently elevated compared with controls (89). Alkaline phosphatase and γ-glutamyl transferase were significantly elevated, as was alanine aminotransferase, a marker of hepatic cell lesion. Thus, women with Turner syndrome seem to have rather distinct alterations in liver function, partly alleviated by sex hormone substitution. These results have recently been corroborated by an Israeli study of young adults treated with either ethinyl estradiol (30 μg) or conjugated estrogens (0.625 mg), both combined with an oral gestagen, in whom liver enzymes were lowered by treatment in comparison with no treatment (303). The enzymes were suppressed more with ethinyl estradiol than conjugated estrogens. We hypothesize that sex hormones may have important protective functions in maintaining normal hepatic function, not only in Turner syndrome, but possibly also in healthy women. It remains to be established in adults, however, whether the effect is mediated by 17β-estradiol or norethisterone (89). The fact that 17β-estradiol exerts the same effect on the indices of hepatic function, irrespective of route of administration, is unexpected in view of the well known first pass breakdown of orally administered estrogens in the liver, where approximately 90% of the drug is metabolized.

**The heart in Turner syndrome**

Much of the increased morbidity and mortality noted in Turner syndrome is attributable to different heart conditions. Some of these are congenital and others are acquired.

**Congenital malformations of the heart**

Turner syndrome is associated with congenital malformations such as coarctation of the aorta, horse shoe kidney and pterygium colli (304, 305), as well as less severe congenital malformations of the heart (306, 307), especially with the 45,X karyotype (Fig. 6) (307–310). The prevalence and the nature of cardiovascular malformations have been described in several studies (Table 3) (306, 307, 311–313). The malformations normally involve only the vessels of the left side of the heart, and show a very characteristic pattern when compared with the general population (311, 312). A recent Italian study found congenital cardiac malformations in 136 of 594 (22.9%) patients with Turner syndrome (311). This figure corroborates previous findings in unselected groups of patients, in whom congenital cardiac lesions were found in up to (20–40%) of patients (307, 312, 314, 315). Notably, a number of patients have more than one structural malformation. In the recent large study by Sybert, it was shown that cardiac malformations are more prevalent among the subgroup of patients with 45,X (39%) than among those with karyotypes that include an isochromosome (Xq) (11–12%) (mosaic or not) (312). In the large Italian study it was also found

![Illustration showing the typical congenital cardiovascular malformations seen in Turner syndrome, i.e. coarctation of the aorta and bicuspid aortic valves. The figure also illustrates the occurrence of aortic dilatation and dissection. Please note that the presence of congenital malformations and/or systemic hypertension is not a prerequisite for the development of dilatation and dissection of the aorta.](www.eje.org)
that cardiac malformations were more prevalent among patients with 45,X (30%) than among patients with X mosaicism (24%) and X chromosome structural abnormalities (iXq, r(X), del(X), etc.) (311). A bicuspid aortic valve is the most common finding, and is seen in 13 – 34% of patients, compared with only 1 – 2% in the general population (316). Coarctation of the aorta is present in 4 – 14% of all patients with Turner syndrome, and predominates in patients with the ‘classical’ karyotype of 45,X. Most patients with aortic coarctation are diagnosed early, due to the relatively severe condition of the aorta. Other malformations affecting the valves have also been reported (Table 3) (307, 311, 312, 314). Clinical follow-up will be necessary in most/all patients. In any case, all patients with either bicuspid valves, septal defects, or other valvular diseases, should be informed of the risk of infectious endocarditis in relation to minor surgery, including dental work, and should be issued proper antibiotic prophylaxis when necessary. Rarely, hypoplastic left heart syndrome (312, 317), which leads to early death if untreated, has also been described (312). The cause of congenital heart defects in Turner syndrome remains unknown. Authors have presented different views on the subject depending on whether they see congenital malformation of the heart in Turner syndrome as (i) a true malformation, linked to the expression of a specific gene or cluster of genes, possibly being X-linked (a gene or genes escaping X inactivation), or (ii) the result of disruption of normal embryonic developmental events, and thus not caused by the karyotype per se. Since patients with 45,X karyotype predominantly are affected by congenital cardiovascular malformations, as well as by lymphedema (and thus webbed neck), deficient development of lymphatics has been proposed as a causative factor. In utero, developing lymph channels distend secondary to failed emptying of jugular lymph sacs into central veins. It is suggested that these distended lymph channels encroach on the heart and great vessels, in this way mechanically inducing congenital heart defects (307, 310, 318). The development of coarctation of the aorta may be due to a combination of maldevelopment of lymphatics and decreased left-sided blood flow in utero (through the aorta), leading to increased flow through the pulmonary artery and the ductus arteriosus, which may induce development of a shelf or flange, leading to later coarctation (309). Recent intriguing observations show that cardiac hypoplasia, along with lung hypoplasia, is widespread among Turner syndrome fetuses recognized due to massive hydrops or large nuchal hygromas (217). The authors examined

### Table 3

Data on congenital malformations are compiled from five studies with apparent unbiased inclusion of patients. Number of affected/total number examined, and percentage are given in the table.

<table>
<thead>
<tr>
<th>Malformation</th>
<th>Miller et al. (351) n = 35*</th>
<th>Dawson-Falk et al. (306), n = 40‡</th>
<th>Gøtzsche et al. (307), n = 179*</th>
<th>Sybert (312), n = 244*</th>
<th>Mazzanti &amp; Cacciari (311), n = 594†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic coarctation</td>
<td>5/40</td>
<td>18/179</td>
<td>34/244</td>
<td>41/594</td>
<td></td>
</tr>
<tr>
<td>Dilated ascending aorta</td>
<td>2/35</td>
<td>5/40</td>
<td>6%</td>
<td>17/594</td>
<td></td>
</tr>
<tr>
<td>Hypoplastic aortic arch</td>
<td>2/35</td>
<td>5/40</td>
<td>6%</td>
<td>17/594</td>
<td></td>
</tr>
<tr>
<td>Bicuspid aortic valve</td>
<td>12/35</td>
<td>7/40</td>
<td>25/179</td>
<td>74/594</td>
<td></td>
</tr>
<tr>
<td>Mitral valve prolapse or regurgitation</td>
<td>2/35</td>
<td>2/40</td>
<td>1/179</td>
<td>53/594</td>
<td></td>
</tr>
<tr>
<td>Interrupted IVC with azygos continuation</td>
<td>1/40</td>
<td>1/179</td>
<td>2.5%</td>
<td>1/179</td>
<td></td>
</tr>
<tr>
<td>Cardiac dextroposition</td>
<td>1/40</td>
<td>1/179</td>
<td>2.5%</td>
<td>1/179</td>
<td></td>
</tr>
<tr>
<td>Aortic valve disease (stenosis/or incompetence)</td>
<td>19/179</td>
<td>14/244</td>
<td>19/594</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial anomalous pulmonary venous drainage</td>
<td>1/179</td>
<td>1/244</td>
<td>17/594</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>0.6%</td>
<td>0.5%</td>
<td>3/179</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrio-ventricular septal defect</td>
<td>12/244</td>
<td>5%</td>
<td>0.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary valve abnormality (stenosis, regurgitation)</td>
<td>2/179</td>
<td>1%</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent ductus arteriosus</td>
<td>2/179</td>
<td>1/244</td>
<td>0.5%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Patients were only examined with clinical exam and echocardiography.
† Patients were examined with clinical exam, ECG, chest X-ray, and transthoracic echocardiography.
‡ Patients were examined by MRI scan and echocardiography. — = not reported.

IVC, inferior vena cava.

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117 Turner syndrome fetuses (32% definitely verified by karyotyping, the rest due to typical stigmata), and found more than 90% of the fetuses to have heart weights below the 2.5 centile. They suggested that myocardial hypoplasia is a primary defect in TS leading to cardiac pump inadequacy, impeding venous return and thereby elevating venous pressure, and that the resulting venous hypertension could lead to diminished lymphatic outflow, and, eventually, to hydrops formation. The phenotype would thus result in intra-uterine death or, if only minimally restricted heart growth was seen, the fetus could survive to birth (217). The study leaves several questions: is cardiac and lung hypoplasia present in live-born TS? Is the hypoplasia linked to other congenital malformations of the heart? Is the hypoplasia actually leading to lymphedema, or is it vice-versa, etc? Other researchers have speculated whether one or more genes on the missing X chromosome are causative. It is supposed that such a gene (or genes) would be one of the many genes on the X chromosome known normally to escape X inactivation (319), a situation leading to haploinsufficiency of the gene (or genes) and the gene-product. The concept of chromosomal imbalance, and thus disturbed pairing during mitosis, has also been implicated (320).

An increase in aortic root diameter, which is a risk factor for developing aortic dilatation and later rupture, is often seen and probably depends on blood pressure (321). However, prospective studies are needed in order to study how the risk of aortic dissection can be reduced. For a more thorough discussion of the heart in Turner syndrome see recent reviews (169, 312).

**Hypertension and ischemic heart disease**

Thirty percent of girls with Turner syndrome are mildly hypertensive on 24-h ambulatory blood pressure monitoring, and 50% have an abnormal diurnal blood pressure profile (172). Women with Turner syndrome have significantly elevated blood pressure compared with an age-matched control group (188), and as many as 50% have clinical hypertension (312, 321). Treatment with sex hormones caused a significant reduction in the 24-h diastolic and the diastolic day pressure, and a near significant fall in systolic day pressure (188). The diastolic night/day ratio was elevated compared with controls and increased even further with HRT, as did systolic night/day ratio. The significance of this is difficult to interpret. It is thus evident that women with Turner syndrome are 'non-dippers', i.e. have a diminished reduction of blood pressure during the night (188). 'Non-dipping' in hypertensive women is a predictor of future cardiovascular events (322). Twenty-four-hour, day, and night heart rates have been found to be significantly elevated in Turner syndrome compared with controls (188), which could be suggestive of the presence of parasympathetic neuropathy. At present, there are no longitudinal studies of blood pressure and hypertension in Turner syndrome. There is a definite need for such studies. Furthermore, it is essential to establish the effect of treatment and to determine which drugs to choose as first and second line treatment.

Previously, ischemic heart disease has not been found with increased frequency in Turner syndrome, despite reports of increased levels of cholesterol (323), increased blood pressure, and congenital cardiac malformations. Recently, in the Turner syndrome population in Denmark (n = 594), however, ischemic heart disease (acute myocardial infarction, and arteriosclerosis) was found to be more frequent in an epidemiological register study of morbidity (8). The RR of disease was increased to 2.1 (95% CI: 1.2–3.4), while hypertension occurred with an RR of 2.9 (95% CI: 1.2–6.0), and cerebrovascular diseases with an RR of 2.7 (95% CI: 1.0–5.3). In a clinical study, we could not detect any difference in measures of lipid status between a group of untreated women with Turner syndrome before treatment and a control group (276). Compensated hypothyroidism is associated with coronary artery disease, and elevated fractions of cholesterol (277, 278). This may help explain part of the increased risk of cardiovascular disease in Turner syndrome (8), since hypothyroidism and thyroid antibody formation is common in Turner syndrome (see above).

**Aortic dissection**

More than 60 case reports of aortic dilatation or dissection, some of which were fatal, have been described (for review, see reference 312). In most cases, risk factors were present. In the general population risk factors for aortic dissection include: (i) systemic hypertension which is present in up to 90% of cases (324, 325), (ii) Marfan and Ehlers-Danlos syndromes are also conditions with increased risk of dilatation, aneurism, and rupture, (iii) congenital bicuspid or unicommissural aortic valves (316, 325), and (iv) coarctation of the aorta (324, 325). In addition, pregnancy, trauma, and iatrogenic-induced trauma are risk factors (324, 325). Usually, but not always, the above mentioned risk factors (other than Turner syndrome) are known to be present in the reported cases of aortic dissection in Turner syndrome (315, 326). At the same time the prevalence and the nature of cardiovascular malformations have been described in several studies (see above) (306, 307, 311–313). Aortic root dilatation, which is a risk factor for later rupture, is often seen and seems to be associated with elevated systolic blood pressure (321). Undoubtedly Turner syndrome should be included in this list of risk factors for aortic dissection. To date, no abnormalities of the aortic wall have been identified in Turner syndrome. Cystic medial necrosis, similar to the changes found in Marfan syndrome, has been described in some (for review see reference 315), but not in all cases. No
biochemical or specific genetic abnormalities have been described in Turner syndrome thus far. Pregnancy is a rare event in Turner syndrome (49, 327). Owing to an increasing number of egg donation programs more patients can be expected to go through pregnancy in the future (58). Due to the pregnancy-associated changes in blood pressure, cardiac work load, etc., the risk of aortic dissection is likely to be increased. Uneventful cases of pregnancy have been reported (327–329); however, fatal and non-fatal cases due to aortic dissection have also been described (330–333). Prospective studies are needed in order to establish the exact risk of aortic dissection, identify patients at an elevated risk, and, if possible, to introduce procedures and/or medicine to lower the risk, both during pregnancy and during normal life.

**HRT and the heart**

The chronic estrogen deficiency affecting many adult women with Turner syndrome is likely to be associated with cardiovascular morbidity. It is generally assumed that treatment with estrogens not only confers cardioprotectivity through a lowering of harmful circulating lipids, but also through direct antioxidant effects (334), a change in the vascular reactivity (335) and its interaction with vascular smooth muscle (336, 337). Part of the increased cardiovascular morbidity and mortality in Turner syndrome could therefore be explained by non-use of estrogens. At present, no long-term studies have assessed the impact of HRT on the heart and on the increased mortality seen in Turner syndrome. It might be speculated that HRT would have positive effects on aortic ‘stiffness’ in the long term (338), but any direct effect on the aortic wall has not been described. Far from all patients receive HRT during adulthood, and many adolescents are introduced to estrogens rather late, in order to achieve the highest adult height. Hypertension is frequent among patients with Turner syndrome (see above), and treatment with female hormone replacement therapy causes a small but significant reduction in the 24 h diastolic and the diastolic day pressure, and a near significant fall in systolic day pressure (188). In hypertensive postmenopausal women, treatment with estrogen, with or without gestagen, has been shown to decrease 24 h ambulatory blood pressure in short-term studies (339). Although lipid abnormalities have been found in a few, but not in most studies (see above), changes have been found during HRT in one study, with a significant, albeit small, decrease in HDL cholesterol, while other lipid variables were unchanged (89, 276), while another study found lipids to be unaffected by HRT (338). Presently, there is a lack of longitudinal, observational and interventional studies of adults with Turner syndrome. It is not known how long-term HRT affects cardiovascular morbidity and mortality. Likewise, the question of when to stop HRT, if ever, is unresolved. As mentioned earlier, three major studies in postmenopausal women have shown that HRT should not be used in this population as primary or secondary intervention against cardiovascular disease (30, 207), or as secondary intervention against stroke (208). However, as discussed above, the applicability of these studies to a population of women with Turner syndrome is dubious.

**Conclusions**

Cardiovascular morbidity and mortality is clearly increased in Turner syndrome. At present, there is no consensus concerning a number of issues: (i) the pathophysiology, especially during adulthood, is not described in any great detail, (ii), there are few long-term follow-up studies, (iii) how aggressively should hypertension be treated, and which drugs to choose as first line drugs? (iv) Especially, the concept of aortic dilatation needs to be dealt with in greater detail. What effect does HRT have during the long term, and how does antihypertensive treatment affect the aorta? (v) How dangerous is child bearing in TS? There are no doubt other issues that are imminent. With our current state of knowledge pertinent clinical guidelines are suggested in the following section.

**Clinical practice of the heart and great vessels in Turner syndrome**

A cardiovascular risk profile should be determined at diagnosis, during adolescence, and in adulthood, and the patient informed about risks and benefits from GH and HRT. Patients should be seen by a cardiologist and an echocardiography performed, together with a clinical examination (Table 4). When pubertal induction is taking place, it may be prudent to perform a new cardiovascular assessment, and likewise in adulthood (4). If any congenital cardiac malformation is present, this should be dealt with properly (see above), relevant examinations and tests performed, and the patient should be followed at regular intervals. Endocarditis prophylaxis should be given in the case of bicuspid aortic valves, and in the case of any surgical procedures having been performed. The potential consequences of GH on the heart and great vessels should be discussed, as well as consequences of HRT, and perhaps especially the consequences of not taking HRT. Based on the available literature HRT is recommended during adulthood. The unsolved problem of who will eventually develop dilatation of the aorta, and thus at great risk of later aortic dissection, leaves the patient and her physician in a situation where repeat echocardiographies, at present, are the only solution. Currently, it is not known how frequent echocardiography (and/or MRI) should be performed. At every visit blood pressure should be monitored. During childhood, normative blood pressure data corrected for height and age should be used, while
Table 4 Clinical practice of patients with Turner syndrome, with respect to the heart and the great vessels.

1. Echocardiography at diagnosis should be performed in all patients. If normal, or near-normal repeat echocardiography should then be performed in adolescence, in adulthood, and probably every 5 years thereafter. A close working relationship with a cardiologist with knowledge of TS is of great value.
2. If congenital cardiac malformations are diagnosed, these should be dealt with appropriately (see text). This includes:
   - operation, if deemed clinically necessary
   - regular clinical exams (echocardiography, MRI scan, blood tests, blood pressure, etc.)
   - prophylaxis for infectious endocarditis (visits to the dentist, minor surgery, etc.)
3. The potential consequences of GH treatment should be evaluated.
4. The benefits and drawbacks of HRT should be discussed with the patient at a relevant age. At present HRT is recommended.
5. Evaluation of the aorta, with emphasis on aortic dilatation, and the subsequent risk of aortic dissection.
6. Cardiac monitoring prior to ART or spontaneous pregnancy, and during pregnancy.
7. Risk of ischemic heart disease.
8. Blood pressure monitoring at every visit to the physician.

ART, assisted reproductive therapy.

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ART, assisted reproductive therapy.

Concluding remarks

The research in Turner women has increased and diversified considerably during the last few years, and much is now known of the natural history of the syndrome with respect to endocrinology and epidemiology. It is clear that glucose intolerance, deficient insulin secretion, thyroid and hepatic abnormalities, android body composition, decreased physical fitness and elevated blood pressure are all part of the syndrome. Thus, several features of the metabolic syndrome (syndrome X) (340) are present in Turner syndrome, and a similarity with the hormonal and metabolic profile of relatives to type 2 diabetic patients is obvious. Women with Turner syndrome have a similar decreased life expectancy (32, 341). This may, however, be due to several reasons: (i) congenital malformations leading to a premature death, e.g. coarctation of the aorta; (ii) increase risk of aortic dilatation with subsequent aortic rupture; (iii) osteoporosis; (iv) endocrinological and metabolic abnormalities; (v) so-called premature aging, which has been postulated to be present in Turner syndrome (342), although we have not been able to confirm the latter in a study of telomere restriction fragment length (TRFL), since TRFL did not differ between Turner patients and controls (343). The reasons for the described hormonal and metabolic abnormalities are not clear. We speculate that one or more genes on the X chromosome may somehow cause some of these abnormalities, although it is as yet impossible to suggest any particular mechanism, and any action may well be indirect. Haploinsufficiency of the SHOX gene may, in addition to being involved in retarded growth (72, 73), be involved in other aspects, like lymphedema, for which a gene recently has been localized (344), and ovarian dysgenesis as well. More likely, however, other yet unknown genes are involved in these processes. A gene effect is suggested by the well known growth retardation during intrauterine life, and the poor thriving during the first year of life (60), leading to an insufficient increase in weight. Recent research has interlinked the circumstances during intrauterine life, i.e. relative deprivation in utero, and the first year of life, and later occurrence of type 2 diabetes, ischemic heart disease, hypertension and obesity (345–349). This may be the basis for some of the aberrations becoming apparent during adult life in Turner syndrome. Thus, many of the hormonal and metabolic aberrations of Turner syndrome could be described as a variant of the ‘prediabetic state’. The surfacing of these aberrations in Turner syndrome may stem from missing or insufficiently expressed genes (and thus extremely interesting genes on the X chromosome).

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